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OCTOBER 1954

VOLUME 94 NUMBER 4

Published Monthly by

AMERICAN MEDICAL ASSOCIATION

535 NORTH DEARBORN STREET • CHICAGO 10, ILLINOIS

Entered as Second Class Matter Jan. 23, 1909, at the Postoffice at Chicago, Under the Act of March 3, 1879. Annual Subscription, $10.00

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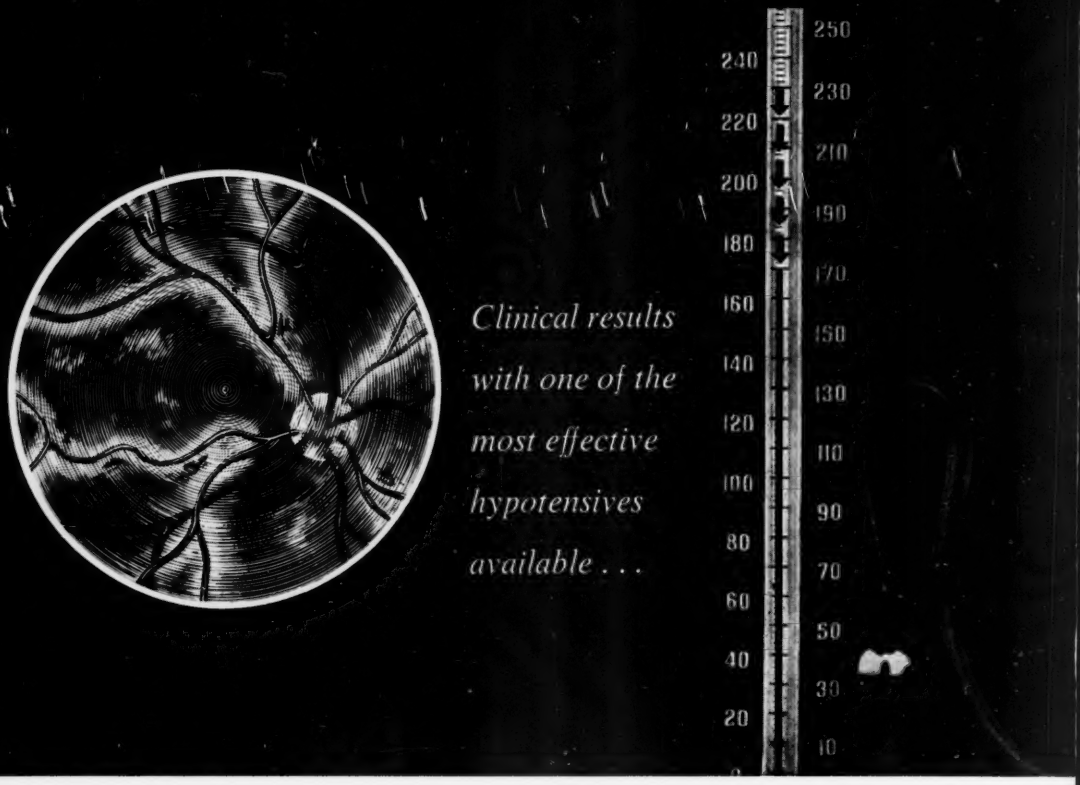

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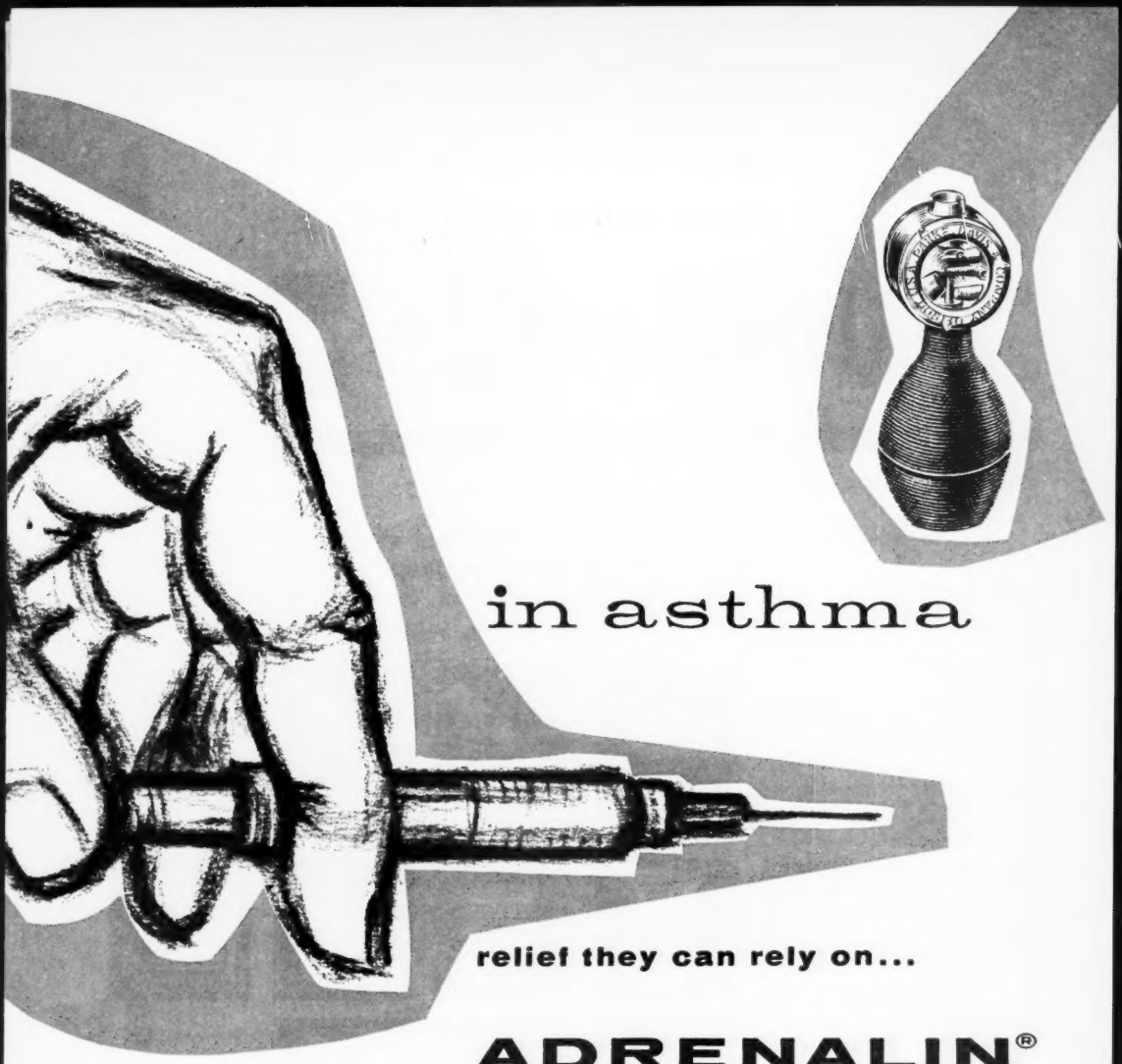

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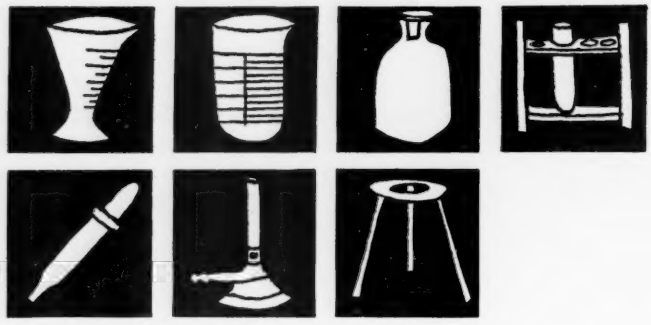

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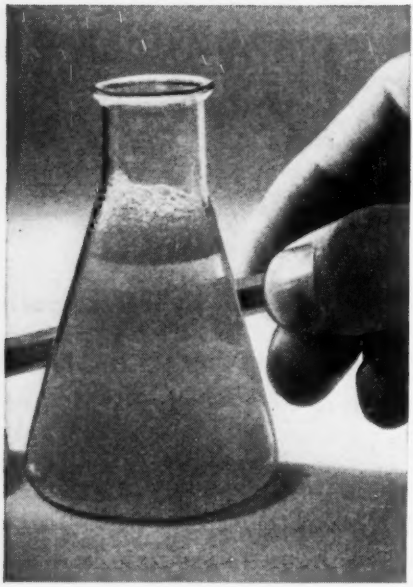

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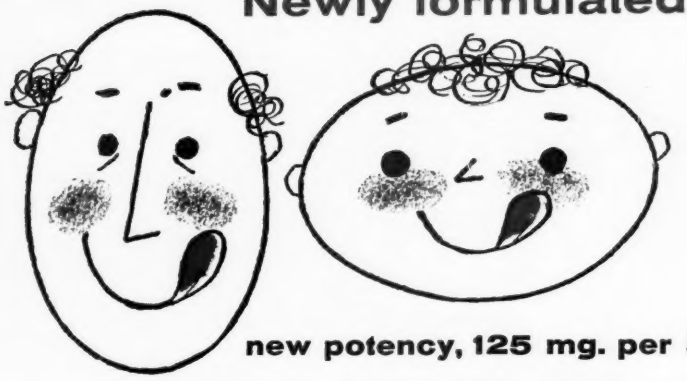

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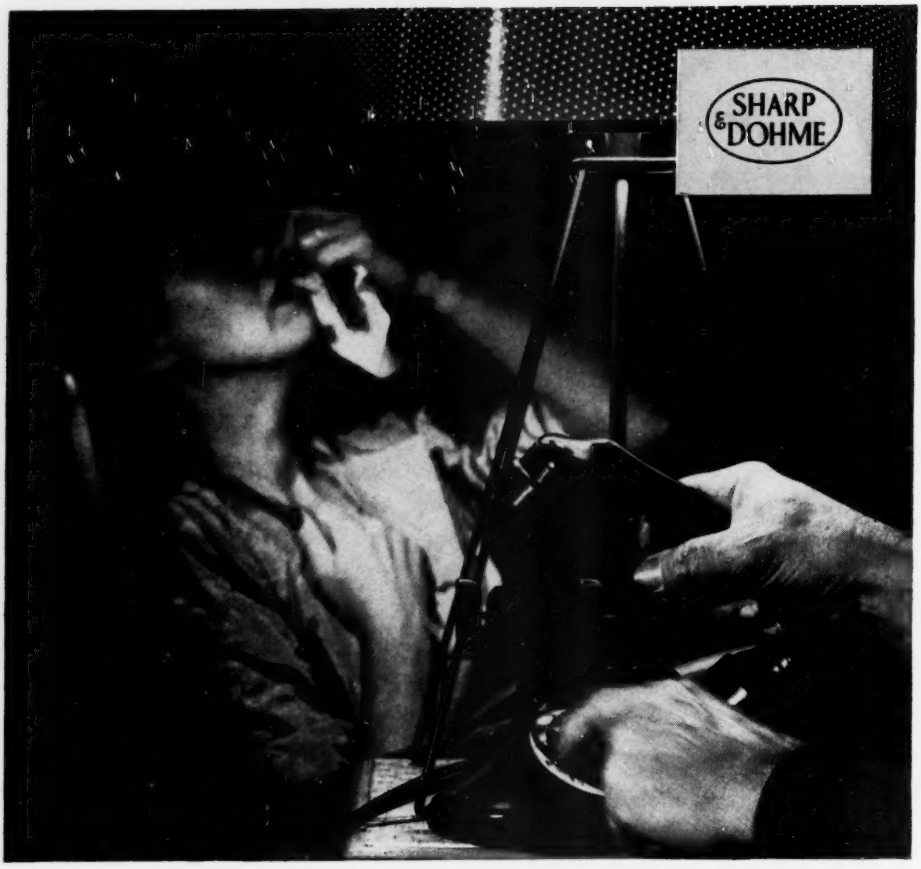

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1. Tulloch, J. and Wright, I. S., *Circulation* 9:823, June 1954.

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Editorials

THE ELECTROCARDIOGRAM AND DISTURBANCES IN ELECTROLYTE BALANCE

EXCELLENT studies * have resulted in one of the most important recent diagnostic developments in electrocardiography, that is, identification of the electrocardiographic pattern of disturbances in electrolyte equilibrium and metabolism. It has been known for many years that ill health of many types can change the T waves, but interest in disturbances in electrolytes has been limited primarily to calcium. The duration of the Q-T interval is known to vary inversely with the ionic concentration of calcium in the plasma, whereas the configuration of the T wave remains unchanged except for duration.[9] Changes in duration of the S-T segment and T wave are primarily responsible for changes in duration of the Q-T interval.[10] Until recently, the U wave was considered to possess no clinical significance and was purely of physiologic interest. However, studies in animals and in man by Winkler and his associates [1]; Nadler, Bellet, and co-workers,[2] and others † have revealed some of the significant changes in the electrocardiogram that can be produced by disturbances in electrolyte metabolism. These observations have resulted in the recognition and description of electrocardiographic patterns which are considered diagnostic of hypokalemia and hyperkalemia. Many observers have recommended that the electrocardiogram be employed for recognition of disturbances in potassium metabolism, especially if facilities are not available for direct measurement of concentration of potassium in the plasma. It has also been advocated that the electrocardiogram be employed to guide therapy for disturbances in potassium metabolism in view of suggestive direct relationship of the level of potassium to a particular electrocardiographic configuration.

Shortly after these clinical advancements were described in the medical literature, they were tested the world over. Previously misunderstood electrocardiograms were recalled and were more accurately interpreted in retrospect. It soon became evident, however, that the electrocardiographic changes described for hypokalemia and hyperkalemia were not as simple as they were first considered. Underlying cardiac disease and alterations in function, which changed the electrocardiogram independently of the effects of alteration in potassium metabolism, complicated the electrocardiographic pattern so that it was often impossible to recognize or evaluate

From the Department of Medicine, Tulane University School of Medicine and Charity Hospital of Louisiana at New Orleans.

* References 1-8.

† References 6 and 11 to 13.

quantitatively the kalemic effects. It was, therefore, necessary to allow for the effects of underlying cardiac disease upon the electrocardiogram during study and management of any problem in potassium metabolism. This was found to limit the usefulness of the electrocardiogram and indicated an even greater need for direct measurements of potassium concentration in the plasma.

Continued interest in the application of the electrocardiogram in diagnosis and management of disturbances in potassium metabolism, as well as simultaneous determinations of the serum concentration, intake, state of acid-base balance, and excretion of other electrolytes, such as sodium and chloride, have revealed electrocardiographic changes in association with disturbances in sodium, chloride, and acid-base balance which resemble those described for potassium effects. Furthermore, Roberts and associates,‡ as well as others,§ have shown that changes in body Na, Cl, pH, HCO_3, Li, Rb, and Cs in man, dogs, and turtles can produce alterations in the electrocardiogram similar to those described for hypo- or hyperkalemia. When the cencentrations of the hydrogen ions, the anions, and the cations were altered, the concentration of the potassium ion was often secondarily altered.‖ These relationships indicate the complexity of the problem.

It has thus become evident that the electrocardiogram must be employed cautiously as an aid in the management of electrolyte problems. It has yet to be established that there is an electrocardiographic pattern characteristic solely of clinical disturbances in potassium metabolism in man. Nevertheless, the electrocardiogram may be employed in the management of hypo- or hyperkalemia when measurements of plasma levels of electrolytes cannot be obtained, provided the interpretations are made cautiously and are properly integrated with the entire clinical picture. Serious difficulties and errors in judgement are not likely to arise if the electrocardiogram is not used as an infallible standard and if its limitations are recognized at all times.

The electrocardiogram more frequently and more reliably indicates the existence, rather than the type and degree, of electrolyte disturbance. For example, when the T wave is low or rounded, the S -T segment low or depressed, the U wave prominent and fused with the T wave, and the "Q-T" interval prolonged,[10] disturbance in electrolyte metabolism is suspected in which K^+ is probably low in the extracellular fluid. In addition, other electrolyte or ionic and other metabolic disturbances may be present. On the other hand, if the Q-T interval is short, the U wave absent, the S-T segment unchanged or deviated from the isopotential line, the QRS complex prolonged with or without the pattern of bundle branch block, and the P-R interval prolonged or the P wave not identifiable, then electrolyte and metabolic disturbances probably exist in which the concentration of K^+ in the extracellular fluid is elevated. However, it is extremely unlikely that the metabolic disturbances in man with disease are limited to potassium or even electrolytes alone. The Q-T or Q-U intervals have not been adequately demonstrated to be prolonged or shortened out of proportion to the change expected from cardiac rate, disease, and

‡ References 11 and 12.

§ References 4, 10, and 13.

‖ References 11 and 12.

the like. It appears that the development or disappearance of a prominent U wave, with its fusion with or loss from a T wave, has been erroneously interpreted frequently as prolongation or shortening of the Q-T interval.[10]

Furthermore, although the electrocardiogram may suggest a disturbance in potassium metabolism, the certainty and extent of such diagnosis cannot be established without the entire clinical data and, usually, measurement of the concentration in the plasma of electrolytes as well. Measurements of the concentration of potassium or electrolytes in the plasma or extracellular fluids alone are not adequate for satisfactory understanding and clinical management of patients with disturbances in water and electrolyte metabolism. Until balance studies and measurement of the major intracellular compartments can be obtained directly, or satisfactorily indirectly by observations upon a readily sampled representative one, our knowledge will remain inadequate and clinical management at least partially empirical.

Whenever possible, changes in the electrocardiogram suggestive of electrolyte imbalance should be followed by study of serum electrolytes as well as daily urinary excretions. Until there is further and more general use of the flame photometer, daily balance recording, and development of practical and reliable intracellular measurements, the full significance of the electrocardiogram and its application to the clinical management of electrolyte disturbances will remain obscure. Obviously, the limiting factor in our electrocardiographic knowledge is concerned primarily with the metabolic processes related to electrolytes. It is necessary to know not only the typical change but, more particularly, the extreme variations as expressed in the absence or presence of coexisting myocardial disease. In the meantime, current knowledge must be applied as intelligently as possible; this can best be accomplished by study of at least the reports referred to herein.

G. E. Burch, M.D.

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SMALL GASTRIC CANCER

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A GASTRIC cancer of small size always arouses the hope that it is more amenable to therapy and possesses a better prognosis than gastric cancer in general. Do the facts justify this hope? Does the small gastric cancer differ in any way from gastric cancers of all sizes? Can it be removed more safely? Are survival rates better for small cancers? Analysis of records of a group of small gastric cancers has in general provided affirmative answers to these questions.

MATERIAL

During the years 1940 to 1945, inclusive, 924 adenocarcinomas of the stomach were treated by gastric resection at the Mayo Clinic. In 226 of these, the greatest diameter measured 4 cm. or less. Thus, gastric cancer of this size accounts for about one quarter of all gastric cancer treated by resection. The importance of this group in the over-all survival rate of patients with gastric cancer is obvious.

For purposes of study, these 226 small gastric cancers were subdivided according to size into four smaller groups, thus providing data about cancers of varying degrees of smallness. The greatest diameter measured 1.0 cm. or less in 18 cases (8%), from 1.1 to 2.0 cm. in 49 (21.7%), from 2,1 to 3.0 cm. in 67 (29.6%), and from 3.1 to 4.0 cm. in 92 (40.7%) of the 226 cases. Lymphosarcomas were excluded from the study so that survival rates would pertain only to adenocarcinomas.

Read before the Joint Meeting of the Section on Gastroenterology and Proctology and the Section on Pathology and Physiology at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 24, 1954.

From The Mayo Clinic and The Mayo Foundation; Section of Medicine (Drs. Comfort and Dornberger), Section of Surgery (Dr. Gray), Section of Surgical Pathology (Dr. Dockerty), Section of Biometry and Medical Statistics (Mr. Gage). From Mayo Foundation; Fellows in Surgery (Drs. Solis and Epperson) and Fellow in Medicine (Dr. McNaughton). The Mayo Foundation is part of the Graduate School of the University of Minnesota.

PATHOLOGIC FEATURES

Of the 226 gastric adenocarcinomas, 223 were ulcerative and only 3 were polypoid. In 11 (4.9%) of the 226 cases the presence of two or more independent cancers recalled that gastric cancer may be multicentric in origin. In another 11 (4.9%) a benign gastric ulcer as well as a gastric cancer were found. Most interesting was the description by the surgeon or the pathologist, or both, of an active duodenal ulcer or the scar of a healed duodenal ulcer in 19 (8.4%) of the cases. In these 19 cases a duodenal ulcer did not ensure the benignancy of the lesion.

Of the 226 cases, 5 (2.2%), 60 (26.5%), 79 (35.0%), and 82 (36.3%), respectively, were graded 1, 2, 3, and 4, according to Broders' method. The combined percentage for Grades 1 and 2 was slightly greater and that for Grades 3 and 4 was slightly less than that for gastric cancers of all sizes.[1] When these small cancers are

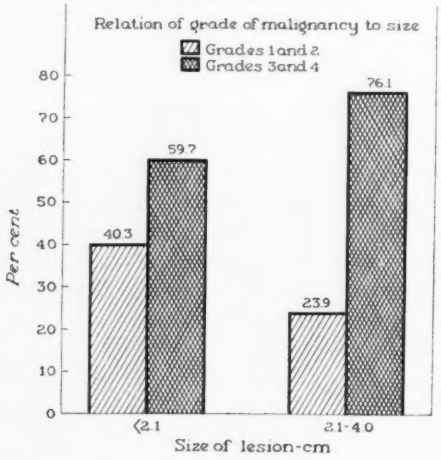

Fig. 1.—Relationship of size of small resectable gastric cancer to grade of malignancy.

divided into subgroups according to size, it is found that the larger-sized ones have a greater incidence of higher grades of malignancy than do the smaller ones (Fig. 1).

Involvement of the lymph nodes was recorded in 43.4% of the cases, a percentage much less than that (58.9%) recorded by Berkson, Walters, Gray, and Priestley [2] in a study of resected gastric cancers of all sizes. When these 226 smaller cancers were divided according to size into the four subgroups, a striking difference in the percentage involvement was seen. Percentages were 11.1, 24.5, 44.8, and 58.7, respectively, when the greatest diameters were 1.0 cm. or less, from 1.1 to 2 cm., from 2.1 to 3 cm., and from 3.1 to 4 cm. The larger the lesion the higher the incidence of lymph-node involvement and, as we have just seen, the greater the incidence of higher grades of malignancy. The correlation between size, grade of malignancy, and presence of metastatic growth is reflected in the five-year survival rates recorded later in this paper.

SEX AND AGE INCIDENCE

Distribution according to sex and age is shown in Table 1. The preponderance of males over females found in all studies of gastric adenocarcinoma also occurred in this group of small gastric cancers; 80.5% of the patients were males, a percentage

TABLE 1.—*Age and Sex Distribution of Small Gastric Cancer*

Age, Yr.	Total		Males		Females	
	No.	%	No.	%	No.	%
20-29	3	1.3	2	1.1	1	2.3
30-39	13	5.8	11	6.0	2	4.5
40-49	48	21.2	41	22.5	7	15.9
50-59	75	33.2	62	34.1	13	29.5
60-69	66	29.2	49	27.0	17	38.7
70-79	21	9.3	17	9.3	4	9.1
Total	226	100.0	182	100.0	44	100.0
Mean	56.1 yr.		55.8 yr.		57.5 yr.	

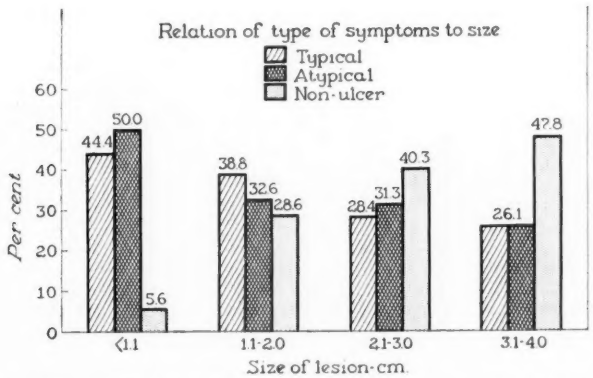

Fig. 2.—Relationship of size of small resectable gastric cancer to type of symptoms.

similar to that (76.6%) recorded by Walters, Gray, and Priestley.[1] A mean age of 56.1 years in our series of small gastric adenocarcinomas is almost identical with the mean age (55.6 years) recorded by Walters, Gray, and Priestley for resected cancers of all sizes. However, the mean age of males in studies of gastric cancer of all sizes has been slightly greater than that of females (56.0 years as compared with 54.0 years, according to Berkson, Walters, Gray, and Priestley, for resected cancers of all sizes, and 56.5 as compared with 55.6 years for resected and nonresected cancers of all sizes, according to Comfort and Vanzant[3]), whereas in this series of small cancers the mean age of females was slightly greater than that of males (57.5 years as compared with 55.8 years). This difference should be erased on analysis of a larger series.

RELATION OF SIZE TO TYPE OF SYMPTOMS

The cases were divided according to symptoms into three groups. Group 1 comprised cases in which the symptoms were typical of peptic ulcer. In this group were 70 (31%) of the cases. Group 2 comprised cases in which the patients complained

of an ulcer type of distress with relief on ingestion of food or alkalies but the symptoms were continuous, lacking periodicity and other details which one expects in a clear history of ulcer. In this group with atypical symptoms of ulcer were 70 (31%) of the cases. Group 3 comprised those cases in which the history did not suggest the presence of an ulcer at any time. This group contained 86 (38%) of the cases. When cases of gastric cancer of all sizes are similarly divided according to type of history, 24.4, 11.2, and 64.4%, respectively, are found in the three groups (Comfort and Vanzant). In small gastric cancer the ulcer and ulcerlike symptoms predominate, whereas in cancer of all sizes symptoms of the nonulcer type occur more frequently.

This tendency toward symptoms of the ulcer type in the smaller and toward symptoms of the nonulcer type in the larger cancers is clearly seen even among these 226 small gastric cancers (Fig. 2). Thus, among gastric cancers whose greatest diameter was 1.0 cm. or less the incidence of symptoms of the ulcer type (44.4%) and of the pseudoulcer type (50.0%) was much greater than that of the nonulcer type (5.6%). As one passes from these very small cancers to those whose greatest diameter measured 3.1 to 4 cm., the incidence gradually changes so that among the

TABLE 2.—*Duration of Symptoms and Type of History of Small Gastric Cancer*

	Total		Type of History					
			Typical Ulcer		Atypical Ulcer		Nonulcer	
Duration of Symptoms	No.	%	No.	%	No.	%	No.	%
<6 mo.	60	26.5	5	7.1	14	20.0	41	47.6
6-12 mo.	28	12.4	5	7.1	11	15.7	12	14.0
1-2 yr.	24	10.6	5	7.1	9	12.9	10	11.6
2-5 yr.	51	22.6	20	28.7	17	24.3	14	16.3
5+ yr.	63	27.9	35	50.0	19	27.1	9	10.5
Total	226	100.0	70	100.0	70	100.0	86	100.0

largest cancers the reverse is true, the incidence of symptoms of the ulcer type (26.1%) and of the pseudoulcer type (26.1%) being less than that of the nonulcer type (47.8%).

RELATION OF SIZE TO DURATION OF SYMPTOMS

Comparison of the duration of symptoms in gastric cancer 4 cm. or less in diameter with that of gastric cancer of all sizes discloses a definite but minor trend toward longer duration in the smaller cancers. Thus, the percentages of cases in which the duration of symptoms was less than 6 months, 6 to 12 months, 1 to 2 years, 2 to 5 years, and more than 5 years were, respectively, 26.5, 12.4, 10.6, 22.6, and 27.9, whereas the corresponding percentages when cases of gastric cancer of all sizes are considered are, respectively, 32.4, 12.3, 16.2, 17.2, and 21.9 (Walters, Gray, and Priestley). The same trend toward symptoms of longer duration for the smaller cancer was noted when the duration of symptoms was correlated with size for these 226 small cancers. This tendency for the duration of symptoms to be shorter for the larger cancers than for the smaller cancers is contrary to what one might expect.

RELATION OF TYPE TO DURATION OF SYMPTOMS

The trend was toward long duration of symptoms when the history was of the ulcer type, whereas the reverse was true when the history was of the nonulcer type (Table 2). Thus, 50% of those with an ulcer type of history had had symptoms for

more than five years, whereas 47.6% of those with a nonulcer type of history had had symptoms less than six months. The distribution of the cases, according to duration of symptoms, of those with atypical ulcer stories was rather even.

TABLE 3.—*Gastric Secretory Activity in Small Gastric Cancer and in Gastric Cancer of All Sizes Compared with the Normal*

	Achlorhydria		Free Acidity, Units	
	%	Diff.*	Mean	Diff.†
Small cancers				
Males	24.6	+9.6	34.4	−9.1
Females	35.1	+15.1	32.8	−0.7
All cancers				
Males	62.8	+44.4	28.2	−14.0
Females	55.9	+35.6	24.6	−8.7

* Percentage difference from incidence of achlorhydria for normal persons of corresponding age and sex.
† Difference from mean free acidity for normal persons of corresponding age and sex.

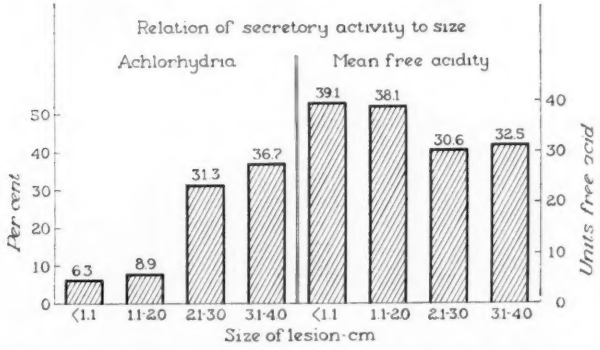

Fig. 3.—Relationship of size of small resectable gastric cancer to gastric secretory activity.

GASTRIC SECRETORY ACTIVITY

Data obtained with the Ewald type of test meal employed at the Mayo Clinic were available in 204 of the 226 cases (167 men and 37 women). Analysis of these data discloses that several well-established facts about gastric secretory activity in gastric cancer in general apply to small gastric cancer.

1. Mean gastric secretory activity is below normal in gastric cancer. In Table 3 are shown the percentages of men and women whose gastric contents did not contain free hydrochloric acid and the mean free acidity of the gastric contents of the remaining men and women, both for this series of small gastric cancer and for a series of gastric cancer of all sizes; also shown are the differences of each from the corresponding values for normal persons of the same age and sex.[4] These data show that gastric secretory activity in small gastric cancer is below normal but much nearer normal than in gastric cancer of all sizes.

2. Gastric secretory activity decreases with increasing size of gastric cancer (Comfort and Vanzant). The incidence of achlorhydria and the mean free acidity

for each subgroup of these small cancers, subdivided according to size, are shown in Figure 3. Even among these small cancers, the incidence of achlorhydria tends to increase and the mean free acidity to decrease with increasing size of the cancer. It is of interest that for gastric cancer 2.0 cm. or less in diameter the incidence of

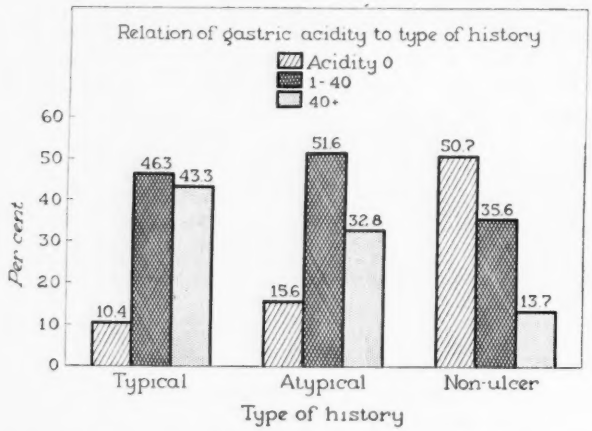

Fig. 4.—Relationship of gastric acidity to type of symptoms in small resectable gastric cancer.

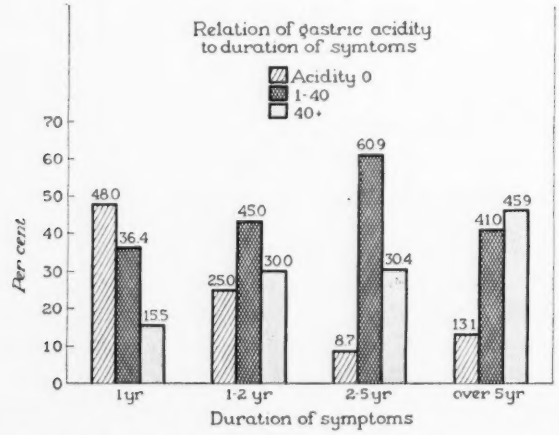

Fig. 5.—Relationship of gastric acidity to duration of symptoms in small resectable gastric cancer.

achlorhydria and the mean free acidity are quite similar to corresponding values for patients with gastric ulcer.

3. Gastric secretory activity varies with type of symptoms (Comfort and Vanzant). The distribution of these small gastric cancers on the basis of gastric secretory activity and the type of history is shown in Figure 4. The incidence of achlorhydria is low and the incidence of free acidity greater than 40 units is high in

those cases in which the symptoms are of the ulcer type, and the converse is true in those cases in which symptoms are nonulcer in type, whereas in those patients with atypical ulcer symptoms, percentages lie in between but are nearer those for the ulcer type. As one passes from the ulcer to the nonulcer type the percentage of cases in which free acidity falls between 1 and 40 units changes irregularly.

Further analysis (Fig. 5) discloses that the shorter the history the higher the incidence of achlorhydria and the lower the incidence of acidity greater than 40 units, whereas the longer the history the lower the incidence of achlorhydria and the higher the incidence of free acidity greater than 40 units.

The highest secretory activity occurs in patients with the smallest cancers, in those with an ulcer type of history, and in those with symptoms of long duration.

ROENTGENOLOGIC DIAGNOSIS

Roentgenologic examination of the stomach was carried out in 222 of the 226 cases. In 8 of the 222 cases an intragastric lesion was not visualized (in 5 of the 8

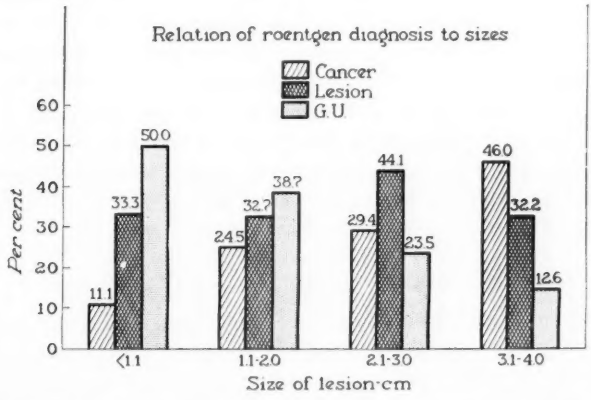

Fig. 6.—Relationship of size of small resectable gastric cancer to roentgenologic diagnosis.

the diagnosis was duodenal ulcer). In the remaining 214, an intragastric lesion was seen. In 5 of the 214 the diagnosis was gastritis or hypertrophy of the pyloric muscle, or both, and in 74, 80, and 55 of the cases the diagnosis was, respectively, carcinoma, lesion, and gastric ulcer. In short, the roentgenologic findings led to a diagnosis of gastric cancer in 33.3% and suggested this diagnosis in 36.0%; in the remaining 30.7% the roentgenologist did not detect features suggestive of cancer. The high percentage of cases in which the diagnosis of cancer was not made merely indicates the difficulty of recognizing the malignancy of lesions of this size and does not reflect on the skill of the roentgenologist. Nor does experience in this series of gastric cancers correctly reflect the over-all accuracy of diagnosis of intragastric lesions, a field in which the accuracy of diagnosis is very high.

The increasing difficulty with which the malignant character of the lesion is recognized roentgenologically as the size of the gastric cancer decreases is clearly seen when these 226 small gastric cancers are subgrouped according to size and the diagnoses are studied (Fig. 6). When the diameter of the lesion was 1.0 cm. or

less, the diagnosis was correctly carcinoma in only 11.1% of cases, whereas when the greatest diameter of the lesion was 3.1 to 4 cm. the percentage was 46. Contrariwise, for the smaller lesions the diagnosis was gastric ulcer in 50% of cases, whereas for the large lesions the diagnosis was gastric ulcer in only 12.6%. The smaller the lesion the lower the percentage of cases in which it was recognized as cancer and the higher the percentage in which it simulated benign ulcer. The percentage of cancers called "lesion" varied relatively little with size in this size range. That the physician does not depend entirely upon the roentgenologist to decide the benignancy or malignancy of small gastric lesions is not surprising.

SURGICAL PROCEDURES AND HOSPITAL MORTALITY RATES

Total gastrectomy was performed in 5, partial gastrectomy in 219, segmental resection in 1, and local excision in 1 of the 226 cases of small gastric cancer. The partial gastrectomy was of the posterior Polya type in 144, anterior Polya in 23, Hoffmeister in 22, Billroth I in 8, Billroth I (Schoemaker modification) in 20, and transthoracic in 2. The over-all hospital mortality rate for the 226 cases was 2.7%. The hospital mortality rate for the 219 partial gastrectomies was 1.8%, a percentage much smaller than that (6.4%) for the 830 partial gastrectomies carried out for gastric carcinoma of all sizes during the same period of time. Also it is a rate quite comparable to that obtaining for partial gastric resection for benign gastric ulcer during these years. It is clear that the risk of surgical removal of small gastric cancer was gratifyingly less than that of gastric cancer in general. The hospital mortality rate for gastric cancer in general and for benign gastric ulcer has fallen since 1945, and that for small gastric cancer may well have done likewise.

SURVIVAL RATES FOLLOWING GASTRIC RESECTION

The 5-year and 10-year survival rates are widely used indices of the effectiveness of the treatment of cancer. The percentage of patients living five years after operation cannot rightly be identified as the proportion of patients cured, for the dual reason that persons who survive that long may well die of cancer and that all who die before that period of time have not necessarily succumbed to cancer. However, when the survival rate for these patients is considered in relation to the survival rate for normal persons of the same age and sex, the resultant rate may be considered as adjusted for deaths not due to cancer.[5]

The survival curve following gastric resection for this group of small gastric cancers is shown in Figure 7; for comparison with this group, a curve for a group of patients with gastric cancer of all sizes and one for a normal population of the same age and sex composition are also shown. The five-year survival rate following gastric resection for patients with small gastric cancers (44.7%) is about 40% greater than that (31.6%) for patients with gastric cancer of all sizes. It also is nearer the rate for the normal population. Somewhere around seven or eight years after operation the curves for the three groups become parallel, indicating that by that time the cancer group has attained a practically normal death rate.

The five-year survival rate following gastric resection for these small gastric cancers varied with size of lesion (Fig. 8). The five-year survival rates for those

with gastric cancer with its greatest diameter measuring 1.0 cm. or less, from 1.1 to 2.0 cm., from 2.1 to 3.0 cm., and from 3.1 to 4.0 cm., respectively, were 82.4, 66.7, 35.4, and 33.3%. The rate (71%) for those gastric cancers whose greatest diameter measured 2.0 cm. or less was more than twice as great as that for gastric cancers of

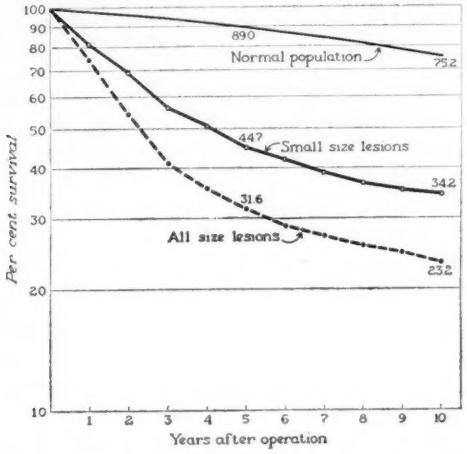

Fig. 7.—Survival rates for patients with small resectable gastric cancer compared to survival rates for patients with gastric cancer of all sizes and for the normal population.

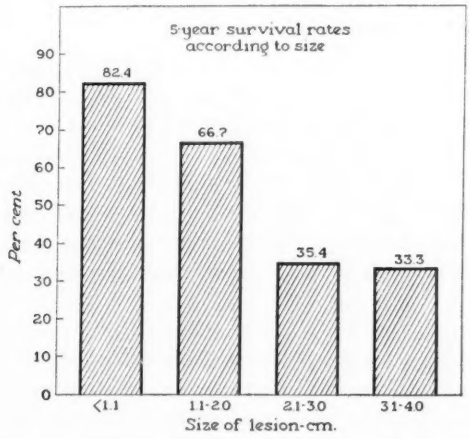

Fig. 8.—Five-year survival rates for patients with small resectable gastric cancer, according to size.

all sizes, but the rate for gastric cancers whose greatest diameter measured from 2.1 to 4.0 cm. was not greatly different from the rate for gastric cancer in general. In this group of small gastric cancers, the smaller the cancer the higher the survival rate.

Berkson, Walters, Gray, and Priestley have shown that survival rates correlate with certain factors.

1. Survival rates are greatly influenced by the presence or absence of metastasis. The five-year survival rate for patients who undergo gastric resection for gastric cancer of all sizes was 48.5% for those without and 18.6% for those with metastasis. This factor appears to have an even more marked effect among patients with small cancers, where the five-year survival rate following gastric resection was 65.6% for those without and 17.9% for those with metastasis.

2. Five-year survival rates correlate unequivocally with grades of malignancy. The lower the grade of malignancy the higher such survival rate following gastric resection and vice versa. Five-year survival rates following gastric resection for cancers of all sizes, graded 1 and 2, graded 3, and graded 4, respectively, were 56.8, 31.4, and 22.8%, whereas the corresponding rates for small gastric cancers of similar grade following gastric resection were, respectively, 60.0, 47.4, and 30.9%. It is interesting that the survival rate was about the same for Grades 1 and 2 in each group but higher for Grades 3 and 4 in the group of small cancers.

3. Five-year survival rates following gastric resection likewise correlate with secretory activity. The higher the mean secretory activity the greater is the survival rate. Among resected gastric cancers of all sizes the only exception to this statement pertains to those patients with achlorhydria, but among small gastric cancers the relationship is consistent both for percentage with achlorhydria and for mean free acidity; thus, the survival rates following gastric resection for patients with achlorhydria and for those whose mean gastric acidity was from 1 to 20, from 21 to 40, and more than 40 units were, respectively, 24.5, 38.5, 55.0, and 59.6%. That a laboratory test performed quite objectively before surgical removal of the cancer should have such definite prognostic significance is remarkable.

The present study also discloses that five-year survival rates following gastric resection vary with the type of symptoms and with the roentgenologic diagnosis. Thus, the five-year survival rates following gastric resection for those patients whose symptoms were ulcer, pseudoulcer, and nonulcer in type were, respectively, 55.9, 47.7, and 33.3%; in cases in which the roentgenologic diagnosis was gastric ulcer, gastric lesion, and gastric cancer, respectively, the rates were 60.0, 42.3, and 32.4%.

Size of lesion, type of history, type of symptoms, gastric secretory activity, and even roentgenologic diagnosis appear to have prognostic significance.

SUMMARY AND CONCLUSIONS

The records of 226 small gastric adenocarcinomas which were 4 cm. or less in greatest diameter and which were treated by resection at the Mayo Clinic in the years 1940 to 1945, inclusive, have been reviewed. Certain significant data have been obtained. These small gastric cancers composed one-fourth of all gastric cancers for which resection was performed in these years. They did not differ from gastric cancer of all sizes in respect to age and sex of patient, but they were associated with a slightly higher percentage of lower grades of malignancy, a lower incidence of metastasis, a higher incidence of symptoms of the ulcer type, and a higher secretory activity than were those of all sizes. These small gastric cancers clinically tended to simulate benign gastric ulcer, and their malignant nature was recognized roentgen-

ologically in only about one third of cases. Most important, the hospital mortality rate was lower and the five-year survival rate higher for these small lesions than for those of all sizes. This numerically large segment of the resectable gastric cancers, difficult to distinguish from benign ulcer, offers a better and a more encouraging prognosis than resectable gastric cancer in general.

Several interesting and important facts evolved when these small gastric cancers were divided into four subgroups according to size and the data were studied.

First, the smaller the growth the lower was the incidence of higher grades of malignancy, the lower was the incidence of metastasis, the higher was the incidence of symptoms of ulcer type, the longer was the duration of symptoms, and the higher was the gastric secretory activity.

Second, the smaller the gastric cancer the more it lacked the roentgenologic features of malignancy and the less frequently was the malignant character of the ulcer recognized. The clinician, being largely dependent upon the roentgenologist for the differential diagnosis of small benign and malignant lesions of the stomach, must be cognizant at all times of the limitations of roentgenologic diagnosis.

Third, hospital mortality rates for small gastric cancer were less than those for gastric cancers of all sizes and were of the same order as those for benign gastric ulcer.

Fourth, the smaller the gastric cancer the higher was the five-year survival rate. This is not surprising, because of the lower incidence of high grades of malignancy and of metastasis among the smaller than among the larger growths.

Fifth, for small gastric cancers, five-year survival rates were higher when the symptoms were of the ulcer type, or of long duration, or when gastric secretory activity was high than when symptoms were of the nonulcer type, or of short duration, or when gastric secretory activity was low. This is understandable when it is recalled that the smaller gastric cancers tend to be related to a higher incidence of symptoms of ulcer type, symptoms of longer duration, and a higher secretory activity as well as to a lower incidence of high grades of malignancy and of metastasis. In short, the small gastric cancer with features suggestive of benign gastric ulcer—ulcer dyspepsia of several years' duration, active gastric secretory activity, and roentgenologic appearance indistinguishable from benign ulcer—has the best prognosis after gastric resection. Unfortunately, this is the gastric cancer most frequently treated medically, in the belief that it is a benign ulcer.

In conclusion, it may be said that the hope that a small gastric cancer is more amenable to therapy and entails a better prognosis than gastric cancer in general is clearly justified by the data obtained in the study of this series of 226 such cancers. An over-all hospital mortality rate of 2.7% after gastric resection for small cancer is much lower than that for gastric cancer of all sizes; the rate of 1.8% after partial gastrectomy is much lower than the rate (6.4%) after partial gastrectomy for cancer of all sizes. A five-year survival rate of 44.7% for gastric cancers with greatest diameters of 4 cm. or less and a rate of 71% for gastric cancers with greatest diameters of 2 cm. or less, respectively, are 40 and 125% greater than the survival rate for resectable gastric cancer of all sizes. The realization for the patient of the high hope found in the smallness of the gastric cancer may be prevented by undue prolongation of medical management, with secondary spread, or assured by prompt surgical treatment.

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EVIDENCE FOR RELATIONSHIP BETWEEN SODIUM (CHLORIDE) INTAKE AND HUMAN ESSENTIAL HYPERTENSION

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THERE are considerable data which indicate that the sodium ion is important or necessary for the development of certain types of experimental hypertension.* So far, to our knowledge, no evidence has been published to suggest that a similar relationship exists with so-called "essential" hypertension in man. A statistical analysis of the data to be presented in this paper, however, indicates that there is a significant correlation between sodium chloride intake and the development of hypertension in man. While the effects of sodium and chloride could not be separated in this study, all the experimental evidence indicates that the sodium ion alone is important, and our interest has centered solely about that ion.

For some years one of us has been intrigued by the lack of significant numbers of persons with hypertension among truly primitive human races. Recently Bays and Scrimshaw [7] have suggested that this may not be uniformly so. Many of the studies cited in their report, however, include groups which can by no means be classed as true primitives. In any event, among a number of others, a virtual absence of hypertension has been found among groups differing as widely in environment as those of the Greenland Eskimo,[8] Australian aboriginal,[9] and mountainous Chinese tribes,[10] as well as the Cuna Indians of Panama.[11] It is commonly averred that the absence of hypertension among such people is due to their simple mode of life, without the strains associated with modern living. Whether this life was simple, carefree, and unassociated with relatively complex decisions is open to question when one reflects upon the dire scarcity of necessities for survival due to rigorous climate and sudden warfare and, perhaps most difficult of all, the taboos and social pressures affecting virtually every aspect of day-to-day living. At least one eminent American anthropologist,† in conversation with one of the authors, expressed grave doubts that the primitive life is as strain-free as has been believed.

It was of interest to find that a factor common to all the primitive groups for which data were available was a low intake of sodium in the diet, estimated from listed foods to average about 1 to 2 gm. per day and sometimes less. Such a level could be attained in the usual western diet only with difficulty, by omitting all added NaCl.

This research was supported by the Atomic Energy Commission.
From the Medical Department, Brookhaven National Laboratory.
* References 1-6 and Dahl, L. K.: Unpublished data.
† Murdock, G. P.: Personal communication to the authors.

The dearth of hypertension among peoples on a relatively low sodium intake suggested the contrary possibility: namely, that hypertension might be more prevalent among groups which on the average had a high sodium intake. The addition of salt to food in large quantities is common practice in western societies generally, and this is not less so in the United States. Further interest in this issue was aroused by finding that among 28 consecutive patients with "essential" hypertension, studied at the Brookhaven National Laboratory Hospital in the past 18 months, only one, a woman of 63 with mild hypertension, denied ever adding salt to food. All five of the patients with severe cerebrovascular disease classified themselves as having added salt to food at table in extremely large quantities for most, if not all, of their adult lives.

With the above factors in mind, early in 1953 it was decided to explore the possible relationship between NaCl intake and hypertension among every member of the Brookhaven National Laboratory staff who reported to one of us for annual physical examination. This would include both scientific personnel and those in the auxiliary services necessary to a large organization. Each person was queried as to his salt intake and classified into one of three groups according to the response: 1. Low intake—these persons could remember no time in their past when they had added salt to food at table. 2. Average intake—these persons added salt to their food only after tasting and finding it insufficiently salty for their palates. 3. High intake—these persons routinely added salt to all foods which are customarily salted and did so before tasting the foods in question. Any person who would have been classified as high or average by past habits but who had changed to average or low intake for any reason was classified as a member of the group with the greater intake to which he had once belonged. No one was classified as being in the low group who acknowledged having ever deviated from strict abstinence at the table.

This classification has certain obvious defects: It does not allow precise estimates of intake; no allowance is made for differences in amounts of salt used in preparing, storing, or cooking of foods, especially certain ones peculiar to the various religious and ethnic groups; no provision is made for individual differences in perception of saltiness, so that the amount of salt added by different persons could vary greatly—and it is even possible that a single person's desires may fluctuate widely; by this classification, a man who adds salt only to eggs which he has occasionally must perforce be classified in the average group, whereas, other things being equal, he undoubtedly would belong in the low category.

However despite the obvious defects listed, it was believed that the conceptual implications of a "low" versus a "high" salt intake might be tested by the present study. Preliminary data suggested that the low intake was about 2 to 3 gm. of Na per day, while the high intake was about five to eight times this amount. The "average" group probably ranged from a relatively low to high intake, and therefore the two extremes of intake were of greatest interest.

It was realized that the group as studied would not be a representative cross section of the population in the United States for at least the following reasons: 1. The preemployment physical examination would eliminate most applicants with severe diseases, and so the population tested was probably healthier than the aver-

age. 2. Due to the employment policies at the Laboratory the age range would be limited to 18 to 65. 3. The sample would consist predominently of males, because the majority of females were seen by a physician of their own sex.

At the time of the examination separate record was made of age, sex, race, nutritional state, and blood pressure. Estimates of nutritional state were based on the undressed appearance, from which each patient was classified as thin, average, overweight, or fat. Although this classification lacked precision and occasional persons were difficult to classify, at this time there appears no need for a more detailed classification. Blood pressure was measured with a mercury sphygmomanometer, with the patient sitting quietly in the upright position with the cuff applied snugly to the arm resting easily on the examiner's table. Systolic and diastolic levels were taken at the first and fourth phases, respectively, as recommended by a committee of the American Heart Association.[12] Hypertension, as defined in this paper, is a level of at least 140 mm. mercury systolic and 90 mm. diastolic. If the blood pressure was not elevated during this examination but previous records of blood pressures of 140/90 or greater were available, this fact was noted as well, and in the final calculations for statistical evaluation of the data any person proved to have had an elevated blood pressure as here defined was classified as a hypertensive. Although not all people with a history of transient elevations of blood pressure will develop sustained hypertension, available evidence suggests that the disease occurs two to three times more commonly among such persons than in those without such transient elevations.[13] Therefore, it would seem even more significant in the final correlation if none of these possible hypertensives were found in the low-intake group.

OBSERVATIONS

The data on 547 consecutive‡ patients are summarized in the accompanying Table. The pertinent observations may be summarized as follows: 1. Forty-three and seven-tenths per cent (239 persons) of this group of American adults routinely added salt to their food at table without prior tasting; 44.4% (243 persons) sometimes added salt after tasting their food, and 11.9% (65 persons) never used salt at table. Although no a priori position had been held, it was a surprise to find so many members in the high-intake category. 2. The mean age and nutritional state were similar among all three groups. However, among the 41 members classed as hypertensives about half were classified as either overweight or fat, whereas among the average- and high-intake groups as a whole this weight distribution occurred in less than one third, and it was found in only 23% of the low-intake group. If it is assumed that the nutritional distribution among hypertensives should reflect that of their respective groups (i. e. average and high intake) as tested by χ squared, the difference may be significant, since $p < 0.025$. 3. Despite the presence of hypertensives in the average- and high-intake groups, the mean blood pressures among the three groups were not significantly different. 4. The presence of hypertension

‡ Data were obtained on three other patients. One male who refused to answer questions proved to have mild schizophrenia; a female, through error, was not queried concerning her salt intake. Neither person had hypertension at this examination or record thereof. A third had proved coarctation of the aorta, with a blood pressure of 180/96; this man was in the average NaCl group.

Data on Salt Intake and Hypertension

	NaCl Intake											
	Low			Average			High			Total, All Groups		
		%			%			%				
	No.	Of Group	Of Total	No.	Of Group	Of Total	No.	Of Group	Of Total	No.	%	
All subjects	65	100.0	11.9	243	100.0	44.4	239	100.0	43.7	547	100.0	
Sex												
Male	54	83.1	9.9	215	88.5	39.3	217	90.8	39.7	486	88.8	
Female	11	16.9	2.0	28	11.5	5.1	22	9.2	4.0	61	11.2	
Age, mean (& S.D.)	36.5 (9.6)	.....	....	35.7 (11.1)	.....	....	35.7 (10.2)	.....	....	...	.....	....
Nutritional state (all persons)	65	100.0	....	243	100.0	....	239	100.0	....	547	100.0	
Thin	8	12.3	...	32	13.2	....	37	15.5	....	77	14.1	
Average	42	64.6	...	135	55.5	....	134	56.0	....	311	56.8	
Overwt	13	20.0	...	55	22.6	....	55	23.0	....	123	22.5	
Fat	2	3.1	...	21	8.6	....	13	5.4	....	36	6.6	
Nutritional state (hypertensives only)	.. ..	.....	....	17	.....	....	24	...	.....	....	....	41	...	.....	....
Thin	..	....	...	2	11.8	....	2	8.3	....	4	9.8	
Average	..	....	...	7	41.2	....	10	41.6	....	17	41.5	
Overwt	..	....	...	7	41.2	....	10	41.6	....	17	41.5	
Fat	..	....	...	1	5.8	....	2	8.3	....	3	12.3	
Blood pressure, mean (& S.D.)												
Systolic	111.6 (8.6)	.....	....	....	...	115.1 (13.4)	.....	....	....	....	114.9 (15.0)	.....	....	....	....	...	...	.....	....
Diastolic	68.5 (7.1)	.....	....	....	...	70.4 (12.3)	.....	....	....	....	70.4 (10.2)	.....	....	....	....	...	...	.....	....
Total hypertensives	0	0.0	0.0	17 (3♀)	7.0	3.1	24 (1♀)	10.0	4.4	41	7.5	
Previous record	0	0.0	0.0	9	3.7	1.6	11	4.6	2.0	20	3.7	
This examination	0	0.0	0.0	8	3.3	1.5	13	5.4	2.4	21	3.8	

or a history thereof as previously defined seemed to be correlated with the classification by NaCl intake. In the low-intake group there was no hypertension; in the average-intake group there were 17 cases (7.0%), and in the high-intake group there were 24 cases (10%). From urinalyses only, there was no evidence of significant renal disease in any of these affected persons.

COMMENT

The correlation between NaCl intake and hypertension among the three groups was tested by the χ-square criterion for significance. With $n = 2$, this analysis revealed $\chi^2 = 7.1$, and therefore $p < 0.03$. This indicates that the probability of such a distribution among the three groups by chance alone is less than 3%. These data do not establish a cause-effect relationship between NaCl intake and the development of hypertension, but this degree of correlation is highly suggestive of such a connection. No explanation is available as to why, among a number of people exposed to a disease-provoking agent, some will and others will not develop the disease in question. Analysis of the data on these people indicated clearly that a high NaCl intake for many years may be found in association with a normal or even low blood pressure. It seems unlikely that members of the low-intake group have been exposed to fewer life-strains than those in the other two groups. Whether the greater number of overweight and fat persons noted above among the hypertensives is important cannot presently be said. The possible relationship of weight and body build to hypertension has been a source of both conjecture and study. In general, it is fair to say that hypertension is found more commonly among persons who are overweight or stocky.§ If it be granted that some minimum level of sodium (chloride) intake must be exceeded for the development of hypertension, it does not follow that such an intake is alone sufficient; it is much more likely that several factors are necessary in appropriate combination. The fact that the stocky and obese persons more often have hypertension than do their slender, asthenic mates may only indicate that, in eating more, these persons use more NaCl, or it is possible that tissue susceptibility to the effects of Na may be an inherited characteristic along with the tendency for a certain body habitus. With highly susceptible tissues, perhaps a moderate or moderately low sodium intake could be followed by the disease; with very resistant tissues, even a high Na intake for long periods of time might not be. Succinctly, the present working hypothesis is that an intake of sodium in excess of an undefined level is a necessary but not sufficient factor in the development of essential hypertension.

The present data do not exclude the possibility that persons predisposed to or with hypertension have a greater drive for NaCl intake and that, rather than bearing a causal relationship to the disease, such an intake is merely a manifestation of the disease.

If, as seems likely, further study confirms the present implications regarding NaCl intake and essential hypertension, it becomes of considerable importance to have more precise estimates of the ranges of actual sodium intakes in these three groups of persons. Such analyses presently are being undertaken.

§ References 14 and 15.

SUMMARY

A group of 547 adults was classified as having low, average, or high NaCl intake at table.

The incidence of hypertension among the three groups was studied.

No hypertension was found among those 65 persons classified as having a low NaCl intake; there were 17 hypertensives among the 243 persons in the average-intake group; 24 of the 239 persons classed as having a high sodium intake had hypertension. As tested by the χ-square criterion, such a distribution would occur by chance in slightly less than 3% of cases.

The hypothesis is proposed that some minimum level of sodium intake must be exceeded for the development of essential hypertension but that, while necessary, sodium is not of itself sufficient for development of the disease.

ADDENDUM

Since submitting the manuscript for publication, the series of observations has been extended to include 897 persons, divided among the salt-intake groups as follows: low, 98; average, 402; high, 397. Hypertension was found in 1, 30, and 40 members of these groups, respectively. By the χ-square test, this distribution in the aggregate could occur in less than 2% but in more than 1% of cases by chance. The distribution by chance in the low-intake group alone has the same low degree of probability. These figures thus further enhance the significance of the earlier data.

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NALORPHINE (N-ALLYLNORMORPHINE)

Practical and Theoretical Considerations

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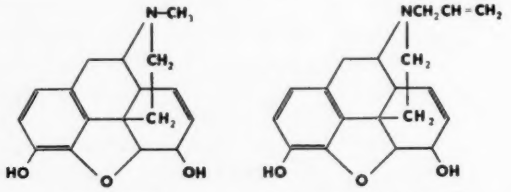
HISTORY

THE HISTORY of N-substituted allyl compounds of the morphine series is both fascinating and instructive. Pohl,[1] almost 40 years ago, became interested in the work of a colleague named Piazza. The latter worker had found allyl compounds to be, in general, irritating to tissues and (under some circumstances) productive of stimulant effects on the respiration. Pohl reasoned that this "stimulant" or "irritant" effect might be "directed towards the central nervous system" by incorporating an allyl group into the opium alkaloids. He accordingly experimented with O-allylnormorphine and N-allylnorcodeine. Investigations in rabbits revealed that the latter drug, while essentially inactive per se, would antagonize the respiratory depression of morphine in rabbit and dog. For over 25 years this exciting fact



MORPHINE *N*-ALLYLNORMORPHINE

Structural formula of morphine and of nalorphine (N-allylnormorphine).

lay buried in the literature. In 1941, McCawley, Hart, and Marsh[2] synthetized what they thought to be N-allylnormorphine (Figure), in a search for a morphine derivative possessing analgesic and narcotic potency but devoid of depressant effect on the respiration. In the same year Hart[3] reported the ability of this preparation to antagonize the respiratory depression of morphine. This material, however, was apparently N-allyl-O-allyl-normorphine, and the actual synthesis of N-allylnormorphine was accomplished by Weijlard and Erickson,[4] in 1942. Unna[5] and Hart and McCawley[6] published extensive investigations of N-allylnormorphine in 1943 and 1944, respectively, establishing beyond question the dramatic ability of this drug to

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This work was supported in part by a grant from the Medical Research and Development Board of the United States Army, Contract No. DA-49-007-MD-92 (made to Dr. H. K. Beecher), and in part by a grant awarded by the Committee on Drug Addiction and Narcotics, National Research Council, from funds contributed by a group of interested pharmaceutical manufacturers.

antagonize many effects of morphine. In 1950-1951 several workers showed that this antagonistic property of N-allylnormorphine applied to other narcotic agents as well.* Despite the generally accepted need for a morphine antagonist, it was not until 1952 that the first † clinical report [10] on the use of this drug appeared in the literature. Since that time, other clinical papers have substantiated the animal data in regard to antagonism of a variety of narcotic agents. The drug (for which the Council on Pharmacy and Chemistry of the American Medical Association has proposed the name nalorphine [11]) is now accepted as an important addition to the therapeutic armamentarium.

PHARMACOLOGICAL EFFECTS OF NALORPHINE

In Animals.—Perhaps the most striking fact about the studies of nalorphine in animals is the apparent relative inactivity of the drug in dose ranges in which morphine produces unmistakable effects. Unna [5] reported that doses of 10 to 20 mg. per kilogram administered subcutaneously to dogs or cats produced no significant changes in behavior other than occasional defecation in the dog within 30 minutes after injection. By contrast, half this amount of morphine produced vomiting, drowsiness, and muscular incoordination in dogs, and restlessness, marked irritability, incoordination, and extreme mydriasis in cats. This same author described the absence of characteristic signs of morphine poisoning (restlessness and the Straub reaction) in mice receiving large doses of nalorphine. With large enough doses, however, the respiration of these animals became labored and irregular and death followed due to respiratory failure. Despite the differences between nalorphine and morphine, the acute $L.D._{50}$ values subcutaneously for the two drugs were almost identical, the major difference between the drugs being the time required for death (15 to 90 minutes for nalorphine; 45 to 180 minutes for morphine). Hart and McCawley,[6] employing the intraperitoneal route, corroborated the similarity in $L.D._{50}$ values for morphine and nalorphine in mice. These authors observed that lethal doses of nalorphine did not produce the sedation seen after morphine and that death was usually preceded by convulsions. They found the Straub reaction to occur after nalorphine but in atypical form. In experiments with 25 mg. per kilogram in two cats, Hart and McCawley corroborated Unna's failure to observe excitement after nalorphine in this species.

The rabbit and dog have been used to study the respiratory and circulatory effects of nalorphine. Unna [5] employed urethan to anesthetize the rabbits for his circulatory studies and described a drop in blood pressure lasting one to three minutes after intravenous doses of nalorphine ranging from 2 to 20 mg. per kilogram. With the higher doses, this drop sometimes amounted to 40 mm. of mercury. Corresponding doses of morphine were less effective in lowering blood pressure. The respiratory rate of such anesthetized rabbits and of unanesthetized rabbits was not changed by these same doses of nalorphine, except for a slight transient increase in rate concomitant with the fall in blood pressure. Doses of 50 mg. per kilogram of nalorphine resulted in death from respiratory failure. Hart and McCawley [6] studied the effect of 5 mg. per kilogram of nalorphine on both respiratory rate and

* References 7-9.

† Actually, a brief preliminary report of this work appeared in abstract form in *Am. J. M. Sc.* **222**:115, 1951.

tidal volume in unanesthetized rabbits. They observed either no change in respiration or a brief intense stimulation of respiration. This stimulation was primarily in regard to rate, with slight decrease occurring in tidal volume. The respiratory minute volume was occasionally increased to twice the control values. A few minutes after injection, however, the respiratory minute volume decreased to "low normal" values, remaining there for one to two hours and then rising to higher values within the normal range. Huggins, Glass, and Bryan [7] anesthetized dogs with barbital sodium and then studied the response of blood pressure and respiratory rate to 20 mg. per kilogram of nalorphine given intravenously. Blood pressure usually fell slightly, returning to normal within 5 to 15 minutes. Respiratory rate either remained unchanged or actually increased for one to two minutes. Tenney and Mithoefer [12] produced acute carbon dioxide narcosis in three dogs. When respiratory minute volume had fallen to half its peak value, 2 and then 4 mg. of nalorphine were given intravenously. No effect on tidal volume or rate was observed.

The effects of nalorphine on the intestine have been studied in the dog. Hart and McCawley,[6] using four trained unanesthetized animals with Thiry-Vella loops, found that 0.5 mg. per kilogram of nalorphine intravenously usually produced a decrease in intestinal tone, with a slight increase in activity (opposite in both respects to the action of morphine in 1.0 mg. per kilogram doses). One dog, however, responded at times with an increase in tone and no change in activity.

Whereas the injection of 2.5 to 5.0 mg. per kilogram of morphine into rabbits was reported by Zauder [13] to result in a rise in blood sugar of 60 to 120 mg. %, "comparable" doses of nalorphine had no effect on the blood sugar level.

Wikler and Carter [14] have studied nalorphine in the chronic spinal dog and have compared it with morphine. In contrast to the marked depressant effects of morphine on ipsilateral flexor and crossed extensor reflexes, nalorphine had only slight effect on these reflexes.

In regard to the analgesic potency of nalorphine there is some disagreement in the literature. Unna,[5] studying the effect of morphine and nalorphine (in mice) on threshold response to electric shock, found only a slight and transient effect with 50 to 200 mg. per kilogram of nalorphine (subcutaneously), and no effect with 10 mg. per kilogram, whereas 5 and 10 mg. per kilogram of morphine raised the threshold by 40 and 80%, respectively, for two to three hours. Smith, Lehman, and Gilfillan [9] restudied the problem in rats and dogs, finding "a weak analgetic action in rats" and no effect on the "pain" threshold in dogs given doses of 2 to 30 mg. per kilogram. Hart and McCawley,[6] on the other hand, using a rat tail-twitch method, found significant "analgesia" from both 3.8 mg. per kilogram of morphine and 1.55 mg. per kilogram of nalorphine. Since there was no difference in the response observed to the two drugs (46% of animals obtaining analgesia in both groups), these authors concluded that the analgesic potency of morphine and that of nalorphine were of the same order of magnitude. Eddy ‡ observed no "analgesic" effect in mice with doses of 2 to 4 mg. per kilogram, but he observed some effect in 15% of animals with 5 mg. per kilogram and in 45% with 50 mg. per kilogram.

In Man.—There is a striking difference between the response of man to nalorphine and the reactions described above in laboratory animals. Whereas the over-all picture in the animal work is one of relative ineffectiveness of sublethal doses of

‡ Eddy, N. B.: Personal communication to the author.

nalorphine in producing detectable changes, the situation in man is remarkable for the ease with which effects (often similar to those of morphine) are produced. For example, Wikler, Fraser, and Isbell [15] have reported the following symptoms in postaddicts receiving 5 to 15 mg. doses of nalorphine (subcutaneously): pleasant relaxation and drowsiness, dysphoria, daydreams, miosis, nausea, giddiness, sweating, ataxia, pseudoptosis, and vomiting. Lasagna and Beecher § have observed the following symptoms in healthy volunteers given 5 to 10 mg. of nalorphine (subcutaneously): shakiness, heavy-headedness, light-headedness, euphoria, dysphoria, headache, nausea, difficulty in concentration and in focusing the eyes, itchiness, apathy, dry mouth, drowsiness, and dizziness. Bodman [16] and two colleagues each took 3 mg. of nalorphine (intravenously). They experienced nausea, giddiness, sweating, shivering, unsteadiness, and heaviness in the head. When nalorphine was given to 15 parturient women by Eckenhoff, Hoffman, and Funderburg,[17] hallucinations, crying, and complaints of muscular weakness occurred in several of the patients. The largest single doses (30 to 75 mg.) have been administered to postaddicts by Wikler and associates,[15] with the production of marked dysphoria, miosis, pseudoptosis, profuse sweating, anxiety, and pressure of thoughts. These unpleasant symptoms were unaffected by morphine but were promptly relieved by 250 mg. of pentobarbital (intravenously). Nalorphine (80 to 120 mg. daily) has been administered to former morphine addicts for 30 days.‖ These addicts did not like the effects of the drug and refused increases in dosage which were offered them. No signs of abstinence were detected following abrupt withdrawal.

The effect of small doses of nalorphine on respiration is impressive. Eckenhoff, Elder, and King,[10] studying five male volunteers, found 5 or 10 mg. of nalorphine (intravenously) to produce no marked or consistent effect on respiratory rate, but there was a uniform diminution in respiratory minute volume, the average depression being 35%. Lasagna and Beecher ¶ observed the effect of 5 mg. of nalorphine (subcutaneously) on the respiratory minute volume response to 5% carbon dioxide inhalation in nine normal volunteers. The magnitude of depression achieved with nalorphine did not differ significantly from that observed with 10 mg. of morphine. Tenney and Mithoefer [12] gave 2 to 10 mg. of nalorphine (intravenously) to normal subjects and patients with respiratory acidosis secondary to pulmonary emphysema. They found an average decrease of 50 and 33%, respectively, in the respiratory response to stimulation with carbon dioxide. The three patients with pulmonary disease all showed marked changes in respiratory rhythm after nalorphine; one of the patients experienced a period of frank delirium. Isbell [18] has reported depression of respiratory minute volume (without particular effect on respiratory rate) with 10 to 30 mg. doses (subcutaneously) administered to postaddicts. With the 30 mg. doses, short periods of apnea occurred, the respiratory pattern resembling very much that seen after 30 mg. of morphine.

§ Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

‖ Isbell, H.: Unpublished data, cited by Isbell and Fraser.[36]

¶ Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

Eckenhoff and associates [10] studied the cardiovascular effects of 5 to 10 mg. doses in their volunteers. These subjects were observed for 15 minutes in the supine position, then were tilted head up at 60 degrees for 15 minutes. In three subjects no marked effect on heart rate or blood pressure was noted. In the fourth subject the blood pressure declined from 125/75 to 75/40 during the 15-minute observation period, and fainting occurred 8 minutes after tilting. The fifth subject fainted 10 minutes after tilting. Wikler, Fraser, and Isbell,[15] employing postaddicts, found 5 to 15 mg. of nalorphine to cause a slight slowing of the cardiac rate and slight elevation of systolic blood pressure. These observers also recorded a slight lowering of body temperature and a marked increase in circulating eosinophile counts following these doses.

Isbell # found nalorphine to raise the pain threshold in man, as measured by the Hardy-Wolff technique. Lasagna and Beecher * administered 5 mg. doses of nalorphine (subcutaneously) to 19 postoperative patients in pain. The frequency of pain relief was less than half that obtained with 10 mg. doses of morphine in these same patients and was of the order of magnitude seen with placebos in other studies of similar patients. Ten milligrams of nalorphine yielded significant analgesia but also produced distressing side-effects (confusion, "nightmares," etc.)

Beal and Schapiro [19] studied the effects of 5 mg. of nalorphine (intravenously) in a patient with intraluminal balloons in the stomach, jejunum, and sigmoid colon. A brief period of increased tonus and contractility was recorded in the jejunum, followed by a period of decreased motility. Gastric and colonic activity were promptly inhibited. These same authors, in another paper,[20] found 5 mg. of nalorphine (intravenously) to have no effect on choledochal pressure, as measured in three patients with choledochostomy tubes.

PHARMACOLOGICAL EFFECTS OF NALORPHINE GIVEN IN CONJUNCTION WITH OTHER DRUGS

In Animals.†—1. Morphine: The ability of nalorphine to prevent or abolish most of the effects of morphine in animals is well documented.‡ Unna [5] was able to prevent or reverse the effects of 5 to 10 mg. per kilogram of morphine in dogs (vomiting, drowsiness, muscular incoordination) and cats (restlessness, irritability, incoordination, mydriasis) by the injection of 10 to 20 mg. per kilogram of nalorphine before or after the morphine. He was also able to raise the $L.D._{50}$ of morphine in mice from 660 mg. per kilogram to 820 mg. per kilogram by prior treatment with 250 mg. per kilogram of nalorphine and to reduce the mortality

Isbell, H.: Unpublished data, cited by Isbell and Fraser.[36]

* Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

† All the drugs reported to be antagonized by nalorphine in experimental animals are listed in Table 1.

‡ The succeeding paragraphs constitute primarily a chronological listing of the work of various investigators, with some sacrifice of strict chronology to grouping of related data. The doses of morphine and nalorphine used are included whenever possible. The route of administration is parenteral in all instances—either intravenous, subcutaneous, or intraperitoneal. Table 2 summarizes these data, as well as those in man.

following 800 mg. per kilogram of morphine from 88% to 28 to 40% by injecting 300 mg. per kilogram of nalorphine (in 2 or 3 divided doses) after the morphine. This same investigator achieved fairly complete antagonism of the "analgesic" effect of 10 mg. per kilogram of morphine in mice by the injection of 100 mg. per kilogram of nalorphine prior to the morphine or 50 mg. per kilogram of nalorphine at the peak of the morphine effect. In urethan-anesthetized rabbits, Unna reported no effect of large doses of morphine on the respiratory rate when this drug was given after nalorphine, and there was a prompt rise in respiratory rate to a normal level when nalorphine was given to rabbits whose respiration was depressed by morphine.

TABLE 1.—*Drugs Antagonized by Nalorphine in Experimental Animals*

	Species	Reference Number
Morphine	Mouse	5, 6, *
	Rat	9, 25, 26
	Cat	5
	Rabbit	5, 6, 13, 22, 23
	Dog	5, 6, 9, 14, 21, 24
Codeine	Dog	7
Dihydromorphinone	Dog	7, 21
Methyldihydromorphinone	Dog	7, 21
Racemorphan	Dog	21
Meperidine	Mouse	8
	Rat	9
	Rabbit	22, 23
	Dog	9, 21
Prisilidene	Dog	21
Methadone	Mouse	9
	Rat	9, 28
	Dog	7, 9, 21
Isomethadone	Mouse	9
	Rat	9
	Rabbit	22
Acetylmethadol	Mouse	9
	Rat	9
	Dog	9
Heptazone	Mouse	9
	Rat	9
	Dog	9
Pentobarbital	Dog	24
Chloralose and urethan	Dog	24

* Eddy, N. B.: Personal communication to the author.

Hart and McCawley,[6] studying respiratory minute volume in unanesthetized rabbits, were able to prevent or abolish the depressant effects of 10 mg. per kilogram of morphine by the injection of 5 mg. per kilogram of nalorphine before or after the morphine. They observed an increase in the $L.D._{50}$ for morphine in mice when nalorphine was added to the morphine. Hart and McCawley also recorded the ability of 0.5 mg. per kilogram of nalorphine to modify the action of 1.0 mg. per kilogram of morphine on the intestine of the dog. Nalorphine given after morphine decreased intestinal tone to subnormal levels, with slight hyperactivity. Since morphine eliminated the decreased tone but not the hyperactivity produced by nalorphine, these investigators felt nalorphine to be incompletely antagonistic to subsequently administered morphine in these circumstances. Gruber and Gruber [21]

antagonized or prevented the increased intestinal tone associated with the injection of 1 to 4 mg. of morphine in trained unanesthetized dogs by the injection of 2 to 5 mg. of nalorphine.

Smith, Lehman, and Gilfillan [9] were able (in rats) to prevent the increase in "pain" threshold, the respiratory depression, and catalepsy ordinarily seen with 6 mg. per kilogram of morphine by the injection of 10 mg. per kilogram of nalor-

TABLE 2.—*Morphine Effects Antagonized by Nalorphine in Experimental Animals and Man*

	Effect	Reference Number
Mouse	Death	5, 6
	"Analgesia"	5, *
Rat	Respiratory depression	9
	"Analgesia"	9, 25
	Catalepsy	9
	Antidiuresis	26
Rabbit	Respiratory depression	5, 6, 22, 23
	Hyperglycemia	13
Cat	Restlessness	5
	Irritability	5
	Muscular incoordination	5
	Mydriasis	5
Dog	Respiratory depression	9, 24
	"Analgesia"	9
	Drowsiness	5
	Depression of spinal reflexes	14
	Muscular incoordination	5
	Miosis	9
	Vomiting	5
	Bradycardia	9
	Hypothermia	9
	Increased intestinal tone	6, 21
Man	Respiratory depression	10, 23, 29-34
	Analgesia	10, †, ‡
	Narcosis	10, 23, 29-33
	"Euphoria"	15
	Miosis	31
	Hypotension	10, 30, 33
	Bradycardia	33
	Nausea	30
	Increased intestinal tone	19
	Increased choledochal pressure	20

* Eddy, N. B.: Personal communication to the author.
† Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.
‡ Keats, A. S.: Personal communication to the author.

phine. They also antagonized (in dogs) the elevation of "pain" threshold, the respiratory depression, bradycardia, hypothermia, and miosis following "small active doses of morphine" by the injection of nalorphine one hour after the morphine.

Snyder,[22] in studies on rabbits in which he was able to observe the respiratory depressant effects of 5 mg. per kilogram of morphine (administered to the mother) on both mother and fetus, brought about a prompt restoration of rate to normal in mother and fetus by the injection of 0.5 mg. per kilogram of nalorphine into the maternal ear vein. He was successful also in reversing the deep respiratory depres-

sion produced with 10 mg. per kilogram of morphine in the newborn rabbit by the injection of 1.0 mg. per kilogram of nalorphine.[23] Vivante and Kao [24] have antagonized morphine depression of respiration in the dog with nalorphine.

Eddy § has been successful in antagonizing the "analgesic" effect of morphine in mice with nalorphine. Orahovats, Winter, and Lehman [25] have had similar experiences in regard to morphine "analgesia" in rats.

Wikler || observed nalorphine to intensify hind limb "abstinence syndromes" in addicted spinal dogs following abrupt withdrawal of morphine. Wikler and Carter,[14] also studying the chronic spinal dog, have antagonized the depressant effects of morphine on the ipsilateral flexor and crossed extensor reflexes with nalorphine. In some experiments, nalorphine not only antagonized or blocked the depressant effects of single doses of morphine on hind limb reflexes but also produced hyperactivity of the latter, with spontaneous "running" movements resembling those seen after abrupt withdrawal of morphine in addicted dogs. Nalorphine also regularly precipitated the general "abstinence syndrome" when injected into dogs "addicted" to morphine for as little as two days. These nalorphine-induced "abstinence" effects resembled those seen after abrupt withdrawal of morphine following weeks or months of addiction to this agent. Morphine was ineffective in terminating such nalorphine-precipitated "abstinence syndromes."

Winter and Gaffney [26] were able to prevent the antidiuretic action of 4 mg. per kilogram of morphine in rats with 2 mg. per kilogram of nalorphine, presumably by blocking the release of antidiuretic hormone. Zauder,[13] by the injection of nalorphine before or after 2.5 to 5.0 mg. per kilogram of morphine, was able to antagonize the hyperglycemic effects of morphine in rabbits.

A failure to antagonize morphine has been recorded by Koppanyi and Karczmar [27] in regard to the convulsant action of this drug in rats and mice. Parenteral injection of 10 to 25 mg. per kilogram of nalorphine, either prior to morphine (300 to 600 mg. per kilogram) or immediately following morphine-induced convulsions, did not significantly decrease mortality or the incidence of convulsions.

2. Other Narcotic Drugs: Nalorphine has also been successfully employed to antagonize a number of other narcotic agents. Huggins, Glass, and Bryan [7] found that 20 mg. per kilogram of nalorphine protected completely against depression of respiratory rate and depth, and death, due to codeine (20 to 40 mg. per kilogram), dihydromorphinone (Dilaudid) (2 to 5 mg. per kilogram), metopon (6-methyldihydromorphinone) (2 to 4 mg. per kilogram), and methadone (2 to 5.0 mg. per kilogram) but not meperidine (Demerol) (10 to 30 mg. per kilogram). It made no difference whether the nalorphine was administered 20 minutes before the narcotic agents or 30 to 45 seconds after respiration had ceased. These experiments were performed on dogs already anesthetized with barbital or chloralose. Radoff and Huggins [8] were able to decrease the mortality rate in mice receiving an $L.D._{50}$ dose of meperidine to 7 to 35% by injecting 0.25 to 20 mg. per kilogram of nalorphine within one minute after administration of the meperidine. Lowest mortality figures were achieved with the largest doses of nalorphine (10 to 20 mg. per kilogram). These authors found that even the 20 mg. per kilogram dose of nalorphine

§ Eddy, N. B.: Personal communication to the author.

|| Wikler, A.: Unpublished data, cited by Wikler, Fraser, and Isbell.[15]

was ineffective if given 5 to 15 minutes after the injection of meperidine. Convulsions, which appeared in 66% of mice treated with meperidine alone, were seen in only 14% of the nalorphine-treated group.

The rise in "pain" threshold, the respiratory depression, and catalepsy observed in rats with 2 mg. per kilogram of methadone, isomethadone, 6-(N-morpholino)-4,4-diphenyl-3-heptanone (Heptazone), or acetylmethadol were prevented by 10 mg./kg. of nalorphine in a study by Smith, Lehman, and Gilfillan.[9] These authors found that nalorphine only partially inhibited the "analgetic" effects of meperidine. They reported that when nalorphine was given to dogs one hour after the administration of "small active doses" of methadone, Heptazone, acetylmethadol, or meperidine, "pain" thresholds returned promptly to control levels and the dogs no longer exhibited symptoms of respiratory depression, bradycardia, hypothermia, and miosis. Nalorphine in doses of 10 mg. per kilogram protected mice and rats against "otherwise fatal doses" of methadone, isomethadone, Heptazone, and acetylmethadol.

Winter and Flataker [28] were able to antagonize the "analgesic" effects of 2 mg. per kilogram of methadone in rats by the injection of 0.5 mg. per kilogram of nalorphine.

Snyder [22] has reported the successful reversal of respiratory depression in rabbit mother and fetus by the injection of 1.0 mg. per kilogram of nalorphine into mothers which had received 10 mg. per kilogram of meperidine and by 0.1 mg. per kilogram of nalorphine in rabbits which had received 1.0 mg. per kilogram of l-isomethadone. In newborn rabbits the respiratory depression induced by 40 mg. per kilogram of meperidine or 6 mg. per kilogram of l-isomethadone was antagonized by 1.0 mg. and 0.6 mg. per kilogram of nalorphine, respectively.[23]

The increased intestinal tone produced in dogs by 0.2 to 2.0 mg. of racemorphan (Dromoran), 2 to 3 mg. of dihydromorphinone, 0.2 mg. of methyldihydromorphinone, 20 to 25 mg. of meperidine, 2 to 2.5 mg. of methadone, or 20 mg. of prisilidene (Nisentil) was prevented or antagonized by 2 to 5 mg. of nalorphine, according to Gruber and Gruber.[21]

There are also two reports dealing with unsuccessful attempts to antagonize the effects of opium derivatives in animals. Unna [5] found that apomorphine produced restlessness and excitement in rabbits, and vomiting in dogs, despite the prior injection of 10 mg. per kilogram of nalorphine. Koppanyi and Karczmar [27] were unable to decrease the mortality or convulsions produced by 30 mg. per kilogram of thebaine in rats and mice by the use of nalorphine.

3. Non-Narcotic Drugs: There is a dearth of information on the effects of nalorphine when administered in conjunction with non-narcotic drugs. Vivante and Kao [24] have produced striking increases in respiratory rate and ventilation by injecting 30 mg. per kilogram of nalorphine into dogs with respiration depressed by pentobarbital. Similar results were obtained with nalorphine when a 1:10 chloralose-urethan mixture was employed as the depressant agent.

In Man.¶—1. Morphine: The first # report of the effectiveness of nalorphine in counteracting the respiratory depressant effects of morphine in man was that of

¶ All the drugs reported to be antagonized by nalorphine in man are listed in Table 3.

A brief preliminary report of this work appeared in abstract form in *Am. J. M. Sc.* **222**:115, 1951.

Eckenhoff, Elder, and King.[10] These workers injected 5 to 10 mg. doses of nalorphine into eight patients who, in the process of undergoing minor surgery under nitrous oxide anesthesia, had received depressant doses of morphine (up to 90 mg.). All patients had a transient doubling or tripling in respiratory rate and increases in minute volume up to 25%. After a few minutes, there was a tapering off of this stimulant effect on minute volume, although the ventilation remained above prenalorphine values for the period of observation. The respiratory rate, however, remained at or close to the peak rate. Second injections were found to be "less effective" than the first, and third injections were "ineffective." A slowing of the heart rate was observed after nalorphine. There was "no marked or consistent effect" on blood pressure if the latter was within normal limits, but a uniform rise of pressure occurred in cases in which the blood pressure had been depressed by the opiate. The blood pressure declined after such initial rises, but it remained above prenalorphine levels. The circulatory effect was slower in onset than the respiratory response, usually taking 5 to 10 minutes to reach a maximum. Eckenhoff and his co-workers reported a delayed lightening of narcosis in these patients but no

TABLE 3.—*Drugs Antagonized by Nalorphine in Man*

	Reference Numbers
Morphine	10, 15, 19, 20, 23, 29-34, *, †
Diacetyl-morphine	42
Dihydromorphinone	29, 39
Pantopon	16, 29
Racemorphan	39-41
Meperidine	10, 16, 17, 29
Methadone	29, 37, 38
Thiopental	31
Thiamylal	31

* Keats, A. S.: Personal communication to the author.
† Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

instances of complete awakening. In four additional patients, 10 to 15 mg. doses of nalorphine (intramuscularly) were followed in 30 minutes by 30 mg. of morphine (intramuscularly or intravenously). Pulse, rate, blood pressure, and respiratory rate were studied in these patients, and no significant changes were observed after the injection of morphine.* The sedative effect of morphine was evident "to a lessened degree."

These authors also reported two additional cases, illustrating the effectiveness of nalorphine in clinical morphine poisoning. One, a 75-year-old man, had been given 50 mg. of morphine (intramuscularly) by mistake. He developed increasingly severe respiratory depression, cyanosis, and apprehension. He received 10 mg. of nalorphine (intravenously), with immediate intense respiratory stimulation and precipitation of anginal pain. Cyanosis disappeared, and apprehension lessened, although Cheyne-Stokes respiration, with periods of apnea, recurred. The second case was that of a 5½-week-old infant who had received morphine (0.13 mg.) and scopolamine (0.1 mg.) as preanesthetic medication. He was operated on under nitrous oxide, cyclopropane, and ether anesthesia. One hour postoperatively, the baby was "depressed," had little muscle tone, and was breathing stertorously

* Presumably, beyond those changes already produced by nalorphine.

at the rate of 22 per minute. Nalorphine (0.1 mg.) was administered intravenously. Within two minutes the muscle tone improved remarkably, respirations became slightly deeper and quiet, and the child moved spontaneously. In 10 minutes the child cried if disturbed, and in 30 minutes he was returned to the ward in good condition.

Another paper from this same laboratory [29] contains the case history of a patient who was given 60 mg. of morphine during minor surgery performed under nitrous oxide anesthesia. Apnea developed, and the patient received 20 mg. of nalorphine (intravenously). There was a striking response, with return of respiratory minute volume and rate to normal levels and a marked analeptic effect. A second patient developed severe respiratory depression and coma after receiving 18 mg. of morphine followed by 100 mg. of meperidine. The injection of nalorphine (10 mg. intravenously) was followed by return of consciousness and great improvement in respiration in a matter of minutes.

Adriani and Kerr [30] have had successful results with nalorphine (intravenously, in doses as low as 2.5 mg.) in 11 patients suffering from respiratory depression, presumably due to morphine.† In six cases of respiratory depression following the use of barbiturates and morphine, the response to nalorphine (as much as 20 mg.) was "not as dramatic." Ten cases of hypotension following preanesthetic medication with narcotics and barbiturates were treated with nalorphine; only 5 of these cases showed a return of blood pressure to control levels. Fifteen instances of postoperative respiratory depression, considered as possibly due to morphine, failed to respond to nalorphine.

Dulfano, Mack, and Segal [31] observed a reversal of respiratory depression and acidosis in an elderly woman, with depressed respiration from 30 mg. of morphine, after intravenous injection of nalorphine (total dose of 25 mg.). Another patient, with depressed respiration, cyanosis, miotic pupils, poor heart sounds, and impaired mental state following repeated doses of morphine and one dose of meperidine, was described as showing improvement in respiration, circulation, and neurological status after 10 mg. of nalorphine intravenously. No further improvement followed the injection of 30 additional milligrams of nalorphine. Marx and Love [32] have reported a case of acute morphine poisoning successfully treated with nalorphine. The patient had received 40 mg. of morphine and 2 mg. of scopolamine inadvertently and responded with improvement in respiration and state of consciousness following the intramuscular injection of 10 mg. of nalorphine. Landmesser, Cobb, and Converse [33] studied nine patients subjected to thyroidectomy. These patients received morphine and scopolamine as preanesthetic medication, were anesthetized with nitrous oxide, and received (in eight out of nine cases) 12 to 56 additional milligrams of morphine during the course of the operation. Rapid intravenous injection of 5 mg. of nalorphine produced an average increase of respiratory rate of 122% in these patients with depressed respiration. Tidal volume increased for a few minutes, then decreased to an average of 27% below prenalorphine values. The minute volume increased and declined in parallel with the tidal volume, but the maintained increase in respiratory rate resulted in a stabilization of minute volume

† In the three cases described in detail, the patients had received single doses of morphine (10 to 15 mg.) and atropine (0.4 mg.). One of these had also undergone surgery, receiving cyclopropane, nitrous oxide, and thiopental (100 mg.) for anesthesia.

at (on the average) 82% above control values. Arterial carbon dioxide content fell an average of 6.5% after nalorphine. Definite increases in blood pressure and pulse rate were observed in five of the nine patients, and analeptic effects occurred in at least six of the patients.

Payne [34] has reported failure of 10 mg. of nalorphine to antagonize the undesirable effects of 15 mg. of morphine in four outpatients and seven healthy males. After nalorphine, three of the four patients showed drowsiness, pallor, sweating, and nausea, with vomiting in two. Nalorphine produced a rise in respiratory minute volume in all the volunteers, but within 10 minutes this stimulant effect had disappeared. Drowsiness, pallor, nausea, sweating and vomiting were also observed in the volunteers.

The clinical use of nalorphine has included the treatment of morphine-produced depression in obstetrics. An infant with severely depressed respiration born of a mother treated with repeated doses of barbiturates and morphine obtained dramatic stimulation of respiration after the injection of 2.5 mg. nalorphine into the umbilical vein, according to Adriani and Kerr.[30] Three similar cases were apparently treated successfully by these authors. Snyder [23] studied 50 multiparous women who received 10 mg. of morphine plus 0.4 mg. of scopolamine one hour before delivery. Infants born of these mothers were given 0.5 mg. of nalorphine intramuscularly 45 minutes (on the average) after delivery. There was marked increase in respiratory rate and amplitude and in body movements within three minutes and crying after six minutes. No unfavorable reactions were observed.

Nausea, increased intestinal motor activity, and spasm of the choledochal sphincter due to morphine have been described as amenable to antagonism by nalorphine. Adriani and Kerr [30] stopped retching and vomiting in a patient who had received 15 mg. of morphine by injecting 5 mg. of nalorphine intravenously. Beal and Schapiro [19] gave a patient 10 mg. of morphine, with resultant marked increase in tonus and contractility of esophagus, jejunum, and sigmoid colon, as measured by intraluminal balloons. Subsequent injection of 5 mg. of nalorphine promptly inhibited the effects of morphine. A second patient, with balloons in the ileum and sigmoid, was subjected to a similar sequence of drugs but showed only questionable response to nalorphine. Schapiro and Beal [20] also measured pressure within the common duct in five patients with choledochostomy tubes. They obtained no characteristic rise in pressure (in two cases) from 10 mg. of morphine when administered after 5 mg. of nalorphine and only a slight increase in a third case. Pressures elevated by this dose of morphine in two patients were decreased somewhat by injecting 5 mg. of nalorphine.

The possibility that the desirable and undesirable effects of morphine may show differential sensitivity to nalorphine antagonism has occurred to a number of investigators. Lasagna and Beecher ‡ have shown that the analgesic effects of 10 mg. of morphine in postoperative patients are not diminished by the addition of 2 mg. of nalorphine. Unfortunately, however, this mixture exhibited as much respiratory depression and ability to produce unpleasant subjective side-effects in volunteers as did 10 mg. of morphine alone. The toxic effects of 15 mg. of morphine

‡ Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

were similarly unaffected by the addition of 5 mg. of nalorphine. Further investigation of this problem, employing a variety of ratios, is continuing. Isbell § has been unable to decrease the respiratory minute volume depression occurring after 30 mg. of morphine by adding 3 to 10 mg. of nalorphine; he did, however, fail to obtain summation of the depressant effects of the two drugs. Cappe and Pallin [35] have claimed that "10 to 15 mg. of nalorphine maximally offsets the respiratory and circulatory depressant effects of 15 mg. of morphine when these drugs are combined." They have observed "intense hypnosis and analgesia" in 75 obstetrical patients who received from 5 to 40 mg. of each drug. The effect on respiratory rate, blood pressure, and pulse rate was "negligible," and labor was considered to have progressed at a normal rate. All infants breathed and cried spontaneously, except for one, who required resuscitation. This study was not a controlled one, and it requires confirmation, as the authors point out.

A particularly dramatic form of antagonism of morphine has been demonstrated in morphine addicts. Isbell and Fraser [36] observed that the morphine abstinence syndrome was intensified by administration of nalorphine during the acute withdrawal period and that this compound blocked the abstinence-alleviating effects of morphine. Postaddicts readdicted to morphine (as little as 15 to 30 mg. four times daily for three to seven days) exhibited "abstinence syndromes" (sweating, mydriasis, tachypnea, restlessness, nausea, muscular aching, yawning, lacrimation, rhinorrhea, gooseflesh) after 15 mg. of nalorphine.[15] Postaddicts given 5 to 15 mg. of nalorphine after 30 to 60 mg. of morphine reported rapid disappearance of "euphoria."

2. Other Narcotic Drugs: Eckenhoff, Elder, and King [10] studied the effects of intravenous nalorphine on five patients showing respiratory and circulatory depression after meperidine (up to 600 mg.) and in two patients showing depression after both meperidine and morphine. These doses of meperidine had been administered during the course of minor operations under nitrous oxide anesthesia. Nalorphine was reported as "effective" in combating such depression. Bodman [16] has reported similar experiences, obtaining stimulation of the respiration (in patients depressed with 50 mg. of meperidine) with as little as 1 mg. of nalorphine. He also restored the respiratory rate to normal with 3 mg. of nalorphine in a patient whose rate had been slowed by 20 mg. of a mixture of hydrochlorides of opium alkaloids (Pantopon) (intravenously).

Two cases of methadone poisoning in postaddicts have been successfully treated by Fraser and co-workers.[37] One patient became comatose and cyanotic, with a respiratory rate of 2 to 3 per minute, after 40 mg. of methadone. Deep tendon, corneal, and gag reflexes were absent. After failure to achieve stimulant effects with 375 mg. of nikethamide, 40 mg. of nalorphine was injected intravenously. Over a period of 23 minutes the respiratory rate rose to 24 per minute. Seventy minutes after the injection of nalorphine the patient was drowsy but could be aroused by vigorous stimulation. Shortly afterward he walked with assistance. The second patient received 30 mg. of methadone some 14 hours after a 20 mg. dose had produced no unusual reaction. This patient also lapsed into coma, with loss of tendon, corneal, and gag reflexes. Respirations were shallow at a rate of 12

§ Isbell, H.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addition and Narcotics, National Research Council, New York, Jan. 23, 1954.

per minute, and the patient was cyanotic. After the intravenous injection of 25 mg. of nalorphine, respirations increased in depth (although the rate was not greatly altered) and within five minutes the patient was completely conscious. Four hours later, a second dose of nalorphine (10 mg.) was administered because of difficulty in keeping the patient awake. A third dose was given 70 minutes later, with progressive recovery thereafter. Finestone and Eksterowicz [38] have also observed reversal of respiratory depression in a case of acute poisoning due to methadone (10 mg.). Nalorphine (10 mg., intravenously) increased the rate and depth of respiration and abolished cyanosis after a variety of analeptics had failed to do so.

Chase, Boyd, and Andrews [39] were successful in antagonizing overdosage of dihydromorphinone and racemorphan in a debilitated 60-year-old man with chronic pulmonary disease. The patient inadvertently received 5 mg. of dihydromorphinone, and became stuporous and cyanotic, with Cheyne-Stokes respirations at the rate of 8 per minute. Within two minutes after the intravenous injection of 2.5 mg. of nalorphine, regular respirations were observed, at the rate of 28 per minute, and cyanosis disappeared. Four hours later the patient's condition had deteriorated again. He received three additional doses (2.5 mg. each) of nalorphine, with improvement in clinical status. It was not until 12 hours after the dose of dihydromorphinone, however, that the patient sat up in bed and talked. Two months later this same patient received 2.5 mg. of racemorphan for relief of pain. He became comatose, with Cheyne-Stokes respirations (7 per minute). Nalorphine (2.5 mg., intravenously) produced an immediate increase in the rate of respiration to 26 per minute, with regular rhythm and good volume. He became responsive to spoken words, and two hours later he was sitting up in bed.

Two cases of racemorphan poisoning treated successfully with nalorphine have been reported by Bornstein, Yorburg, and Johnston.[40] These patients had been overdosed, inadvertently, with 50 and 25 mg. of racemorphan, respectively. The first patient, after unsatisfactory results with various analeptics, was given 20 mg. of nalorphine (intravenously). Respirations increased, cyanosis disappeared, and within 10 minutes the patient (previously comatose) was able to answer questions clearly. Following this improvement, however, the patient became agitated and talked incessantly in a repetitious manner. She next became drowsy (although able to talk rationally) for a period of time, and finally she lapsed back into respiratory depression. Nalorphine was given again (10 mg., intravenously), with prompt but slight increase in respiratory rate and dilatation of the (miotic) pupils. An hour after this second dose of nalorphine the patient became restless and disoriented and remained so for about one-half hour. The second patient reported by these authors became bradypneic, cyanotic, and disoriented despite supportive measures and analeptics. Nalorphine was administered intramuscularly "in doses of 5 mg." In 20 minutes the patient began to shake violently and have slight spastic movements of arms and legs. Respirations increased, and in six hours her condition had returned to that existing prior to racemorphan administration.

Domino, Pelikan, and Traut [41] have reported the successful use of nalorphine (5 mg., intramuscularly) in a patient severely depressed after a total of 10 mg. of racemorphan. Respiratory, general depressant, and analgesic effects of the latter were antagonized, in that sequence. A second episode of racemorphan-induced depression in the same patient a few days later was treated with 2.5 mg. of nalorphine (intramuscularly), with similar results.

Eckenhoff, Hoffman, and Dripps [29] have successfully treated cases of respiratory depression due to Pantopon, dihydromorphinone, and methadone. Two patients received 10 and 20 mg. of nalorphine to counteract respiratory depression following 80 and 100 mg. of Pantopon, respectively. Both had an increase in respiratory rate and minute volume; there was also a slight analeptic effect in one and a rise in blood pressure from 106/70 to 150/96. Three patients received 20 mg. of nalorphine after having received 6 to 8 mg. of dihydromorphinone. All three had increases in respiratory rate, minute volume, and blood pressure, with slight to moderate analeptic effect. One of these patients received a second dose of nalorphine (15 mg.) 40 minutes after the first, with further improvement. Two patients received 15 to 20 mg. of nalorphine for respiratory depression occurring after 19 to 20 mg. of methadone. One of these patients, who had suffered only mild depression, exhibited a slight increase in minute volume, with no significant change in respiratory rate. The other patient, with marked depression of respiration, had a doubling of respiratory rate and minute volume. Both patients showed a moderate to marked analeptic effect.

A single case of acute diacetyl-morphine (heroin) intoxication has been reported as showing favorable response to nalorphine.[42] An unknown quantity of diacetylmorphine had produced coma, miotic pupils, and respiratory arrest in a 22-year-old woman.‖ Intravenous nalorphine (5 mg.) initiated respirations, dilated the pupils, and restored consciousness within one minute.

Eckenhoff, Hoffman, and Dripps [29] have investigated the usefulness of nalorphine in obstetrical complications due to meperidine. They arbitrarily assigned mothers to the following categories, based on clinical grounds: (1) not depressed, (2) mildly depressed, and (3) moderately or deeply depressed. The average doses of medication were 100 mg. of meperidine, 0.4 mg. of scopolamine, and 0 to 0.2 gm. of secobarbital in Group 1; 150 mg. of meperidine, 0.6 mg. of scopolamine, and 0 to 0.2 gm. of secobarbital in Group 2; and 200 mg. of meperidine, 0.7 mg. of scopolamine, and 0.2 to 0.3 gm. of secobarbital in Group 3. Almost all the mothers received nitrous oxide, and 40% also received ether as supplemental anesthesia. Nalorphine (10 mg.) was given intravenously to approximately half the mothers, usually about 10 minutes before birth of the infant. There was no significant difference (in regard to time required to gasp or to establish cry) between the untreated control infants and the nalorphine-treated infants among those babies born of mothers who were not depressed or only mildly depressed. In the moderately to deeply depressed groups, however, statistically significant improvement was demonstrated in both parameters in the nalorphine-treated cases.

Eckenhoff, Hoffman, and Funderburg [17] have described their experiences in antagonizing neonatal narcosis in 1,100 cases. Approximately half of the mothers received 10 mg. of nalorphine (intravenously); the rest received isotonic sodium chloride solution. A small group of infants was studied whose mothers had not received narcotic sedation. Nalorphine may possibly have had a slight deleterious effect in these infants, since 7% were subjected to resuscitation in the nalorphine group, as opposed to none in the untreated group. In addition, the times required to gasp and initiate respiration were slightly longer in the nalorphine-treated group which received nitrous oxide anesthesia during delivery than in the corresponding

‖ The urine and vomitus of this patient also showed the presence of barbiturate.

controls. In infants born of mothers who had received "opiate"¶ sedation, with regional anesthesia, nalorphine did not significantly shorten the time required to gasp or breathe, but it apparently decreased the number of cases receiving resuscitative measures (*2% vs. 25%* in the controls). In the entire group of mothers receiving "opiate" sedation plus general anesthesia (nitrous oxide or ether) there was no perceptible advantage from nalorphine treatment. Only in the heavily sedated parturients, who were given nalorphine prior to delivery and nitrous oxide during delivery, was there a significant decrease in the average times required by the infants to gasp and establish respiration. Even in this presumably "optimal" group, *17%* of the nalorphine-treated infants were resuscitated, as opposed to *27%* in the control group. The most striking effects of nalorphine in this study occurred in 12 apneic infants given 0.1 to 0.2 mg. in the umbilical vein. Eleven of these babies had "excellent" responses. Bodman [16] obtained "good results" from 0.5 mg. of nalorphine given to each of two infants delivered "unexpectedly quickly after Pantopon."

Wikler, Fraser, and Isbell [15] have been able to precipitate clear-cut "abstinence syndromes" by the subcutaneous administration of 15 mg. of nalorphine to post-addicts receiving 10 mg. of methadone or 15 mg. of diacetyl-morphine (Heroin) for as short a period as 2 to 3 days. Isbell [43] has reported similar precipitation of the "abstinence syndrome" in dihydromorphinone and acetylmethadol addicts. Isbell, Fraser, and Wikler [44] have also induced "violent abstinence" with nalorphine in patients receiving 3-ethylmethylamino 1:1-di-2[1] thienyl-but-1-ene. Postaddicts receiving 5 to 15 mg. of nalorphine after 5 to 20 mg. of methadone or 15 mg. of heroin reported rapid disappearance of "euphoria." [15]

3. Non-Narcotic Drugs: Eckenhoff, Elder, and King [10] were unable to combat "depression" produced by thiopental (two patients), cyclopropane (two patients), or ether (three patients) with nalorphine. Eckenhoff, Hoffman, and Dripps [29] observed no analeptic or respiratory effects after 30 to 40 mg. of nalorphine in two patients depressed from secobarbital (150 to 200 mg.). Adriani and Kerr [30] administered 10 mg. doses of nalorphine to six patients with respiratory depression due to barbiturates; the nalorphine was "totally without effect." Dulfano, Mack, and Segal,[31] however, were successful in antagonizing barbiturate-induced depression in two patients. One patient developed shallow respirations (8 per minute), hypotension, miosis, and stupor after 1.5 gm. of thiopental. Nalorphine injection (20 mg., intravenously) was followed within five minutes by a rise in blood pressure and respiratory rate and volume and an increase in the activity of deep tendon reflexes. There was some decrease in respiration within the next five minutes, although the state of consciousness showed rapid improvement. A second dose (20 mg.) of nalorphine brought the respiratory rate up to the level seen after the first dose. Recovery was uneventful. The second patient had received thiamylal (Surital) (2 gm.) and nitrous oxide for anesthesia during a radical mastectomy. Postoperatively she was hypotensive, cyanotic, comatose, and breathing shallowly at the rate of 7 per minute. After 15 mg. of nalorphine (intravenously) respiratory rate doubled (with increase in depth), blood pressure rose, and cyanosis disappeared. Within 15 minutes cyanosis recurred as the respirations decreased in rate and depth. A second dose of nalorphine (20 mg., intravenously) was followed

¶ The only "opiate" used in this study was meperidine. Secobarbital and scopolamine were also administered.

by a rise in respiratory rate and exchange and in blood pressure. Within 10 minutes the patient began to respond to questioning, and further recovery was uneventful.

It should be pointed out that in many of the cases in the literature described as "morphine depression," "meperidine depression," etc. which responded favorably to nalorphine barbiturates had also been given (see above). In some of these cases it is difficult to exonerate the barbiturate with certainty and equally difficult to be sure of the nature of the nalorphine antagonism.

Gravenstein # injected 10 mg. of nalorphine into a patient severely depressed from overdosage of paraldehyde, with no improvement in cardiovascular, respiratory, or neurological status.

THEORIES OF MECHANISM OF ACTION OF NALORPHINE

There has been a tendency to think of the antagonistic effects of nalorphine in terms of a competition, between this drug and structurally related drugs, for cell sites.* The reversal (or prevention) of depression and other morphine effects has thus been conceived as the substitution of the effects (or lack of effects) of nalorphine for those of morphine and similar depressants. Much of the animal work can be satisfactorily explained by this simple concept, since doses of nalorphine which have been successfully employed to antagonize narcotics in species other than the human are per se essentially devoid of effect.

There are difficulties, however, in postulating such a hypothesis to explain all the available data. For example, the demonstrated effectiveness of nalorphine in antagonizing a variety of narcotic drugs only remotely related to morphine structurally and in reversing depression due to barbiturates raises strong doubts about the importance of the structural resemblance between nalorphine and drugs antagonized by it. It is true that all the drugs antagonized by nalorphine are respiratory depressants and may be acting at similar cell sites. There is "displacement" of these drugs by nalorphine in a physiological sense, and this may be true in a literal sense as well (regardless of dissimilarities in structure). We lack sufficient data to confirm or deny this lattter possibility, although recent neuropathological studies † suggest that there is competition between nalorphine and a variety of narcotics for cell sites on certain myelinated neurons.

The theory that one is always simply substituting the effects of nalorphine for those of other drugs, however, is suspect on other grounds. In man, for example, within the dose range usually employed in treating narcotic depression, nalorphine is, milligram for milligram, as depressant to the respiration as morphine. Unless one proposes a marked differential sensitivity in individuals to the effects of the drug responsible for depression and the effects of nalorphine, many of the reported cases are difficult to explain. In addition, there is a distinct impression to be derived from the work of a variety of observers that the effectiveness of nalorphine is directly related to the magnitude of depression present or to the magnitude of the dose of narcotic. Eckenhoff and co-workers,[29] for example, were successful in demonstrating significant improvement in infants with depressed respiration born of mothers with moderate or deep depression but not in those born of mothers who

Gravenstein, J. S.: Personal communication to the author.

* In place of "for cell sites," one could substitute "for susceptible enzyme systems," "in metabolic processes," etc. without affecting the subsequent argument.

† Scharenberg, K.: Unpublished data, cited by Seevers and Woods.[60]

were mildly depressed (who had received less narcotic on the average). This failure is particularly interesting, since the number of cases was actually larger in the latter group (which should have helped in the establishment of significance) and since the average time required to gasp was not different in the untreated controls in both groups (suggesting that no major difference in the therapeutic problem was posed in the two groups). In contrast to the success of nalorphine in the severely depressed cases reported in the literature is its failure in the studies of Isbell,[43] Lasagna and Beecher,‡ and Payne.[34] Isbell has reported that "when [nalorphine] is given after a dose of morphine which does not cause rather marked depression, we have observed that the effects [of nalorphine] synergize with those of morphine." Lasagna and Beecher failed to demonstrate significant antagonism of the relatively minor respiratory depression and other side-effects of 10 mg. of morphine by 2 mg. of nalorphine, and of 15 mg. of morphine by 5 mg. of nalorphine. Payne was unimpressed by the ability of nalorphine (10 mg.) to reverse untoward effects of morphine in patients and subjects given 15 mg. of the latter drug. In animals there is a parallel situation in the work of Eddy § and of Wikler and Carter.[14] Eddy found the "analgesic" effects of morphine in mice to be more effectively antagonized by nalorphine when larger doses of morphine were used than when smaller doses were used. Wikler and Carter demonstrated antagonism of morphine by nalorphine most clearly in the case of spinal reflexes when the latter were markedly depressed by morphine.

It is still possible to reconcile these findings (on relation of magnitude of depression to nalorphine effect) with the substitution theory. One possibility is that when a minor degree of depression is present, the substitution of another minor degree of depression (i. e., that of nalorphine) will produce no discernible effect, whereas in cases of major depression a similar substitution of a minor depression will yield a net improvement in status which is demonstrable. A second possibility is that the larger the dose (or effect) the greater the saturation of cell sites and the less chance for occupation by nalorphine molecules of unoccupied sites rather than displacement of narcotic molecules from occupied sites.

A different explanation, however, for the available data has been suggested.‖ This postulates that the antagonistic effects of nalorphine may at times be dependent on the release of the mechanisms responsible for physical dependence. In support of such a theory can be marshalled the following evidence: 1. The apparent relationship between effectiveness of nalorphine as an antagonist and the magnitude of dose of morphine is reminiscent of a similar relationship between degree of physical dependence and the size of dose of narcotics.[45] 2. Under certain circumstances the results of nalorphine injection are unequivocally something more than a mere substitution of its effects for those of another drug. An example is the precipitation by nalorphine of "withdrawal symptoms" in addicted humans and dogs, since there is no evidence to suggest that "withdrawal symptoms" ever result from the admin-

‡ Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

§ Eddy, N. B.: Personal communication to the author.

‖ This theory was presented by the author at a meeting of the Committee on Drug Addiction and Narcotics, in New York City, Jan. 23, 1954. Subsequently the author has learned of the independent formulation of the same theory by Dr. A. Wikler.

istration of nalorphine per se in the absence of narcotic. 3. Abstinence symptoms are in many respects opposite in action to the usual effects of morphine, and include restlessness, dilatation of the pupils, increased blood pressure, and tachypnea. These phenomena are described after nalorphine injection in successfully treated clinical cases reported in the literature. Of particular interest is the finding that tachypnea induced by nalorphine injection in humans with physical dependence to morphine is often striking and more marked than that seen in the ordinary course of morphine withdrawal.[15] 4. Orahovats, Winter, and Lehman [25] have demonstrated an increased ability of nalorphine to antagonize the "analgesic" effects of morphine in rats as the rats developed tolerance (and presumably physical dependence) to morphine. 5. Houde,¶ at an institution dealing almost exclusively with cancer patients, has observed an interesting difference in the amounts of nalorphine required to produce reversal of narcotic depression due to overdosage in various patients. In clinical depression in patients not tolerant to narcotics 45 mg. of nalorphine may be required to produce results achieved by 1 to 3 mg. of nalorphine in similarly depressed patients who have received narcotics over a long period of time and are partially tolerant to them.

Consideration of this theory (i. e., the dependence of the antagonistic effects of nalorphine on the "unmasking" of physical dependence phenomena) would seem to require at least two assumptions: (1) the relatively rapid development of physical dependence (at least in those persons who obtain stimulant effects from nalorphine) and (2) some independence of tolerance and physical dependence, since cases of clinical depression following narcotics are by definition not particularly tolerant to the narcotics. The first assumption is supported by several bits of information. Wikler and co-workers [15] have shown that postaddicts may develop definite physical dependence after as few as nine injections of morphine, methadone, or diacetyl-morphine. In dogs, successive single injections of morphine and nalorphine may result in "abstinence" phenomena.[14] Similar "rebound" reflex hyperactivity may occur after recovery from marked depressant effects of single large doses of morphine.[46] In volunteer subjects receiving single "therapeutic" doses of morphine or diacetyl-morphine it is not uncommon to have a secondary unpleasant reaction described, suggestive of increased central nervous system irritability, after an initial "relaxation" or drowsiness. These reactions are described as "shakiness," "tenseness," or "grouchiness" and are at times accompanied by actual tremor.# A postoperative case has recently been described * in which the responses to nalorphine suggest that development of physical dependence had occurred after five injections of morphine. Nalorphine (5 mg.) after the third dose of morphine in this patient produced no untoward effects, but after the fifth and seventh doses of morphine nalorphine produced marked apprehension, tachypnea, and generalized severe pain. This pain was interpreted as due to the unmasking of a nonspecific heightened susceptibility to peripheral stimuli rather than to a simple antagonism of persistent analgesic effects of previously administered mor-

¶ Houde, R. W.: Personal communication to the author.

Lasagna, L.; von Felsinger, J. M., and Beecher, H. K.: Unpublished data.

* Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

phine. Keats † observed a similar precipitation of generalized pain after nalorphine in a patient who had received a single dose of morphine postoperatively.

The second assumption can also invoke fragmentary data for support. In the spinal dog, Wikler and Carter [24] have precipitated "abstinence syndromes" with nalorphine at a time when no evidence of tolerance to certain depressant effects of morphine was apparent. The work of Orahovats and co-workers [25] with rats also suggests a lack of parallelism between tolerance and physical dependence, since an increased effectiveness of nalorphine as an antagonist of morphine appeared after one and two weeks of morphine treatment, when only minor tolerance to morphine was evident. It is of interest that their work with postaddicts and spinal dogs has led Wikler, Fraser, and Isbell [15] to infer that physical dependence processes are ordinarily "masked" by the "narcotic" effects of opiates responsible for miosis, respiratory depression, and sedation.

Still another theory to explain some of the nalorphine data can be proposed in terms of a "synergism" between nalorphine and morphine in the sense of magnifying certain morphine effects by antagonizing other effects. For many years the "dual action" theory [47] has occupied a prominent role in discussions on the mechanisms of morphine action. This theory visualizes the simultaneous existence of depression and stimulation in the central nervous system following the injection of morphine. There is a good deal of evidence to support the statement that morphine is both a stimulant and a depressant, and one may conceive of stimulation after morphine as a "primary" effect of morphine rather than a cellular "counteradaptation." If nalorphine is capable of antagonizing primarily certain depressant (i. e., "narcotic") effects of morphine, the results of its administration will depend on the completeness of this antagonism plus the magnitude and nature of the ordinarily "masked" excitant effects which can be made manifest. The apparent relationship between dose of narcotic and effectiveness of nalorphine described above might thus be explained as the "unmasking" of a greater degree of stimulation. Certain of the descriptions of nalorphine-treated cases in the literature are compatible with the precipitation of marked stimulant effects (e. g., the "agitation" and the "violent shaking and spastic movements" in the two cases of Bornstein, Yorburg, and Johnston [40] and the "excited and uncooperative" behavior described in the report of Adriani and Kerr [30]). The failure of Koppanyi and Karczmar [27] to antagonize morphine convulsions suggests an inability of nalorphine to block certain stimulant effects of morphine. The intensification by morphine of "abstinence" effects precipitated in the spinal dog by nalorphine [14] also fits in with this concept. A recent report,[48] however, indicates that nalorphine administered to morphine-tolerant rats caused sedation, a picture similar to the effect of morphine withdrawal in this species. This finding seems to support the concept of "unmasked counteradaptations" rather than the theory of antagonism of depressant effects.

Certain interest in the role played by cholinesterase inhibition in morphine antagonism has been aroused by the demonstration that a variety of anticholinesterase drugs may antagonize certain effects of morphine.‡ Nalorphine is a powerful inhibitor of cholinesterase activity.[53] Somewhat confusing, however, is the fact that morphine is likewise a potent inhibitor of cholinesterase.§

† Keats, A. S.: Personal communication to the author.

‡ References 49-52.

§ References 53 and 54.

It must be emphasized that a discussion of the mechanism of action of nalorphine in antagonizing morphine and other drugs is at the moment more an intellectual exercise than anything else. Ultimate mechanisms of action of most drugs (if not all) are currently almost impossible of detection because of the primitive status of our knowledge of cellular pharmacology. The preceding paragraphs have been presented to demonstrate the difficulties involved in the facile exposition of any single hypothesis of nalorphine action to explain all the available evidence and to stimulate the acquisition of data which may shed light on this important but paradoxical antagonism of depressant drugs by another depressant drug.

USE OF NALORPHINE IN DIAGNOSIS, THERAPEUTICS, AND RESEARCH

Diagnosis of Addiction.—The diagnosis of narcotic addiction may be extremely difficult at times. It is now established that the injection of nalorphine may precipitate acute "withdrawal symptoms" in patients who have been taking as little as 10 mg. of either morphine or methadone four times daily for seven days.[55] Persons addicted to diacetyl-morphine, dihydromorphinone, or acetylmethadol have also shown "withdrawal symptoms" after nalorphine injection. The recommended procedure [55] is to give 3 mg. of nalorphine subcutaneously to a suspected addict followed by an additional 5 and 8 mg. at 20-minute intervals if no typical "abstinence syndrome" develops. If definite "abstinence symptoms" are precipitated, it is suggested that 15 to 30 mg. of either morphine or methadone be given, although these symptoms usually disappear spontaneously after three hours. In at least one addict, the injection of 30 mg. of nalorphine has resulted in extreme "withdrawal effects" which were unresponsive to repeated large doses of morphine.[15] The "abstinence syndrome" subsided spontaneously about two hours later; the patient remained amnesic for the entire episode. In any event, the use of nalorphine as a diagnostic tool is not to be undertaken without due consideration of the problems involved, both medical and medicolegal.[55] Of interest is the statement that the daily intake of meperidine must exceed 1,600 to 2,000 mg.‖ for "abstinence symptoms" to be precipitated by nalorphine.

Therapy of Narcotic Depression.—The experience in both animals and man indicates undeniably the effectiveness of nalorphine in antagonizing at least certain cases of narcotic depression due to overdosage or to exceptional sensitivity to usual doses. Reversal by nalorphine of respiratory depression, hypotension, or coma following morphine, dihydromorphinone, methadone, diacetyl-morphine, meperidine, Pantopon, and racemorphan has been reported. In certain instances, the clinical situation described was of such seriousness that supportive therapy might conceivably have failed to tide the patients over. It is thus possible that nalorphine is at times little short of life-saving, although the unequivocal superiority of nalorphine to optimal supportive treatment in decreasing mortality has not been demonstrated.

There are, however, suggestions that nalorphine is not always effective. Adriani and Kerr's [30] 15 cases of postoperative respiratory depression and 5 cases of hypotension which failed to respond to nalorphine would have qualified as instances of narcotic depression if they had improved after nalorphine administration. I ¶ have

‖ References 43 and 55.

¶ Lasagna, L.: Unpublished data.

seen several cases of respiratory depression after narcotics which have failed to show any improvement after 10 to 20 mg. doses of nalorphine. One of these cases showed respiratory depression and stupor on three separate occasions, with the only common denominator being the administration of 8 to 10 mg. of morphine one to two hours prior to each episode of depression. On the second and third episodes, the patient received 10 and 20 mg. of nalorphine, respectively, with no significant clinical improvement. Payne,[34] after injecting 10 mg. of nalorphine (intravenously) into four patients and seven subjects who had received 15 mg. of morphine, detected no marked or clinically useful antagonism. How widespread such experiences have been is impossible to say, particularly since "negative" reports have an unfortunate habit of not getting into the literature.

While it is tempting to dismiss all such "failures" as instances of depression not induced by narcotics, or examples of inadequate dosage of nalorphine, such a cavalier attitude is probably unwarranted. It is, of course, imperative to consider other causes of depression in every case, particularly in the event of "unsuccessful" treatment with nalorphine. What shall constitute an adequate trial with nalorphine is a difficult question. A dose of 10 mg. (parenterally #) can in most instances be recommended as a safe exploratory dose, but it is not easy to delimit the total amount of nalorphine to be injected if no favorable response occurs after such a dose. As little as 1 mg. and as much as 45 mg. have been successfully used to treat cases of narcotic depression. It has been suggested that larger doses of nalorphine are more effective than small doses, although the evidence is not clear-cut in this regard. The data available on the effects of nalorphine per se show that the drug can cause respiratory depression which is severe. The possibility that a diagnosis of narcotic poisoning is incorrect and that nalorphine administration may merely superimpose an iatrogenic depression on the already existent depression must give even the enthusiastic physician pause. Although some have stressed the innocuousness of the drug in instances in which it failed to produce improvement, Payne [34] has reported unpleasant and prolonged effects after its administration. It is suggested that the reliability of the diagnosis of narcotic depression, the clinical state of the patient, and the response to pilot doses of nalorphine serve as bases for decision as to the total dosage of nalorphine to be employed in an individual case.

Use in Obstetrics.—The use of nalorphine in obstetrics is really a facet of the general problem of the treatment of narcotic depression. There is good reason to believe that nalorphine, given either to the mother shortly before delivery or to the infant after delivery, is capable at times of reversing depression due to morphine or meperidine. The doses which have been employed for this purpose are 10 mg. (intravenously) for the mothers and 0.1 to 2.5 mg. for the infants. The latter have been treated both intramuscularly and by injection into the umbilical vein. In obstetrics, however, as in the treatment of narcotic poisoning in adults, there are instances of failure of nalorphine to antagonize narcotic-induced depression. Thus Eckenhoff and his colleagues [29] were unsuccessful in establishing significant differences between control and nalorphine-treated groups when mothers were not depressed, or mildly depressed, by meperidine and barbiturates, and even

Most reported cases have been treated intravenously, although there is probably no particular advantage to this route, except perhaps in regard to speed of action.

in a selected series [17] of 86 nalorphine-treated "moderately or deeply depressed" cases (where significant improvement could be demonstrated), 17% of infants required resuscitation, as opposed to 27% in the control group. These results strongly suggest that there is little reason to assume the arrival of a pharmacological millennium in obstetrics merely because of the availability of nalorphine. While certain cases of depression will undoubtedly be helped by this drug, it is not clear that the net gain in the majority of patients warrants increased or promiscuous use of depressant analgesics in childbirth. The accent should more appropriately be placed on the prevention of postnatal depression in infants rather than attempts at correction of such depression. The place of nalorphine in obstetrics remains to be defined; at the moment the drug is probably best reserved for depressed infants born of mothers treated with narcotics and not responding to simple resuscitative measures.

Treatment of Depression Due to Non-Narcotic Drugs.—The situation in regard to the use of nalorphine in cases of poisoning due to barbiturates and other non-narcotic depressants is a confused one. Despite the failure of Eckenhoff and co-workers [29] and Adriani and Kerr [30] to reverse barbiturate-induced depression, the experience of Dulfano and co-workers [31] in man and of Vivante and Kao [24] in dogs indicates that under appropriate circumstances nalorphine may act as a barbiturate antagonist. Whether nalorphine possesses any advantages over picrotoxin, pentylenetetrazol, and other analeptics in such cases is a moot point. Indeed, Nilsson's work in Copenhagen,[56] in an institution where large numbers of severely poisoned barbiturate cases are seen yearly, has revived older arguments against the use of analeptics in such cases. Treating a total of almost 5,000 cases with purely supportive measures, he has reported a mortality rate of 2% or less for four consecutive years.* Such a performance leaves little room for improvement.

The depressions induced by ether, cyclopropane, or paraldehyde have reportedly been uninfluenced by nalorphine.

Use of Morphine-Nalorphine Mixtures.—The need for a potent analgesic devoid of depressant effects and addicting properties has long been appreciated. The data available at present preclude the formulation of dogmatic statements as to whether mixtures of morphine and nalorphine will provide a partial or complete solution to this problem. The preliminary observations of Lasagna and Beecher,† Isbell,‡ and Cappe and Pallin [35] are somewhat contradictory, and the problem deserves and requires further investigation.

A major difficulty may lie in the capacity of nalorphine, in 5 to 30 mg. doses, to produce respiratory depression, nausea, vomiting, etc. The mere substitution of the effects of nalorphine for those of equal quantities of morphine would hardly provide clinical results preferable to those obtained with morphine alone. The question of antagonism of morphine analgesia in man by nalorphine is another potential obstacle to the discovery of useful mixtures of the two drugs, although preliminary investigations suggest that nalorphine may actually produce a significant

* Nilsson, E.: Personal communication to the author.

† Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

‡ Isbell, H.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York, Jan. 23, 1954.

analgesic effect in man. If the data of Lasagna and Beecher § are confirmed, one possible usefulness for mixtures of morphine and nalorphine in rations of 10:1 or 5:1 is suggested by the finding that such mixtures produce a milder degree of physical dependence than do equivalent doses of morphine in postaddicts.‖ Such an effect appears unimportant in ordinary short-term use of morphine, where euphoria is infrequent and addiction liability low. In long-term use of narcotics, this effect may be of some importance, in the prevention of either psychological or physical dependence. An obvious advantage to an effective analgesic mixture of this sort would be the deterrent effect it would have on the illicit traffic in morphine by persons with access to legal sources of the drug (drug stores, hospital pharmacies, etc.). (It is important to remember that doses of nalorphine of the order of 1 to 5 mg. are capable of precipitating acute withdrawal symptoms in addicts taking large quantities of morphine daily.) Against these advantages must be weighed (1) the cost of such mixtures as compared with morphine alone, (2) the absence of data on the analgesic activity and addiction liability of such mixtures under a variety of conditions (such as chronic use, for example), and (3) the relatively minor role played by "bootlegging" of morphine obtained from legitimate sources in the entire addiction problem.[57]

Use of Nalorphine in "Basic" Pharmacology.—The application of nalorphine to research in what might be entitled "basic" pharmacology may yet yield the most abundant scientific harvest of all. The exciting findings of the research group at Lexington, Ky.,¶ are perhaps the best example of what has been uncovered to date and what may be learned in the future. Their work has given further insight into physical dependence in the addicted animal and man by revealing phenomena which are usually manifested only after the withdrawal of narcotics. They have advanced evidence which suggests that in the case of some narcotics (methadone, for example) the normally mild withdrawal symptoms occurring after abrupt termination of drug injections may give a false notion of the degree of physical dependence actually extant while an addict is on the drug. The work of this group in the spinal dog indicates the distinct possibility that tolerance and dependence need not always coexist. The rapidity with which physical dependence to narcotics seems to develop in man has been another interesting result of their application of nalorphine.

Of considerable significance, also, is the existence (in nalorphine) of a drug which mimics so closely many effects of morphine, both subjective and objective, yet seems to present a dissociation of these properties and that of dependence. The search for the long-desired "ideal" potent analgesic has thus received impetus after many years of depressing failure in this field.

PREPARATION OF OTHER N-SUBSTITUTED COMPOUNDS

Pohl [1] originally studied O-allylnormorphine and N-allylnorcodeine, with primary emphasis on the latter compound. In the years since then nalorphine, N-allylnorheroin, N-allylnormeperidine, and many other compounds have been

§ Lasagna, L., and Beecher, H. K.: Unpublished data, read before the Thirteenth Meeting of the Committee on Drug Addiction and Narcotics, National Research Council, New York Jan. 23, 1954.

‖ Isbell, H.: Personal communication to the author.

¶ References 14 and 15.

synthesized. The evidence to date suggests that a requisite for antagonistic activity is the presence of a 3-carbon alkyl side-chain on the nitrogen atom.# Published reports * are available only on levallorphan (1-3-hydroxy-N-allylmorphinan), and suggest that this drug is a nonspecific antagonist of opiate-induced respiratory depression in man and animals. Whether any of these compounds will ultimately prove more useful than nalorphine, either as antagonists or as analgesics, is a matter for speculation at the moment. It would be of interest to know whether particular allyl derivatives are more efficacious in antagonizing their parent compounds than allyl derivatives of other drugs. Another question is whether "antagonistic potency" of allyl derivatives applies to all modalities affected by a particular narcotic drug or whether there is a significant differential sensitivity of various narcotic effects to antagonism. Studies of these problems in experimental animals and man are obviously in order.

SUMMARY

The available data on nalorphine are reviewed. Its effects in the experimental animal and in man are described and contrasted. The abundant evidence for antagonism, by this agent, of a variety of drugs (narcotic and non-narcotic) is cited. The difficulties involved in formulating a simple theory to explain all the available data on this antagonism are pointed out. Finally, the present status of nalorphine in diagnosis, treatment, and research is described.

Eddy, N. B.: Personal communication to the author.

* References 58 and 59.

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APPARENT FAILURE OF CHICK-EMBRYO-ADAPTED HEPATITIS VIRUS TO IMMUNIZE AGAINST NATURAL VIRUS

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PROGRESS in the knowledge of infectious hepatitis has been slow owing to the fact that the disease has not been transmitted to an experimental host. Colbert [1] has recorded the negative data obtained in many hosts by different workers. In 1950 Henle and co-workers [2] reported the propagation of the agent in tissue culture and embryonated hen's eggs. Although no lesions in these hosts were recognized, inoculation of volunteers with passage materials thought to contain hepatitis virus produced a mild disease, described as hepatitis without jaundice.[3] Other investigators * have been unable to confirm this. In addition Henle, Drake, Henle, and Stokes [4] have described a skin test for infectious hepatitis, using as antigen amniotic fluid prepared from chick embryos thought to be infected with the virus of hepatitis (IH) and irradiated by ultraviolet light. This was seen to give allergic reactions in a high percentage of persons who had recovered from infectious hepatitis. However, the antigen is not produced with regularity; it is unstable, and nonspecific reactions are not infrequent.[5] It has also been suggested [5] that the use of the skin test might cause the development of immunity in a previously susceptible person. Because of the importance of these observations, confirmation was sought (*a*) by infection of volunteers with egg-passage materials by a different group of observers (C. I. L. and G. S. M.) and (*b*) by challenge, with natural virus, of any volunteers who developed signs of disease, to determine their degrees of resistance after convalescence.

MATERIALS AND METHODS

Egg-Passage Materials Thought to Contain IH Virus.—The chick-embryo-adapted hepatitis virus was prepared by one of us (G. H.). Two strains were used, designated as Frost 13th and Chicago 12th chick embryo passage. The former represents a combination of two passage

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This work was carried out under the sponsorship of the Commission on Liver Diseases, Armed Forces Epidemiological Board, and was done in part under contract with the Office of Naval Research.

* Kilham, L., and Murray, R.: Personal communication to the authors; Gordon, I.: Personal communication to the authors.

lines: A. The Akiba strain was passed originally 10 times in minced chick embryo tissue culture, 3 times amniotically, and once in volunteers. B. The New Lisbon strain was carried initially through 10 tissue culture and 1 human passages. Acute stage sera from one volunteer each infected with Lines A and B were then given simultaneously to another group of volunteers by the oral route, primarily in order to determine whether the disease induced by the passage materials could be transmitted serially in man. One of these volunteers (Frost) developed a somewhat more pronounced illness than seen in the previous groups (total serum bilirubin 1.6 mg. per 100 cc. and sulfobromophthalein [Bromsulphthalein] retention 36%. Serum taken from this patient during the acute stage was then used to initiate further amniotic passages. Amniotic fluid of the 12th transfer was injected amniotically, and after an incubation period of six days at 36 C. the embryos, amniotic membranes, and yolk sacs were emulsified in a Waring Blendor. Three milliliters of the resulting 50% suspension and 2 ml. of amniotic fluid were fed to each volunteer. Although the Frost strain represents possibly a mixture of two viruses, they appear to be closely related, as indicated by previous cross immunity tests in volunteers.[2] The amniotic passage series of the Chicago strain was initiated with acute stage serum from a volunteer infected with a stool suspension of an intestinal carrier (IH virus) found in an orphanage in Chicago.[6] Amniotic fluid of the 11th passage was injected into chick embryos, and the material for feeding was prepared as described for the Frost strain.

Natural (Unadapted) Hepatitis Virus (Akiba Strain).—This virus was originally obtained from an outbreak in a summer camp[7] and was maintained by passage in volunteers. Plasma obtained from eight volunteers during the acute stage of illness was employed in the challenge experiment. Aliquots of these samples were pooled for preliminary titrations of infectivity in volunteers. However, the variable results obtained by Dr. Gordon and by the Philadelphia group suggested the presence of antibodies in one or more of the plasmas, and consequently it was decided to feed the samples separately at half-hourly intervals, using 0.3 ml. of each sample, in order to avoid neutralization. Thus, the challenge dose was unknown.

Skin Test Antigens.—These were prepared by Dr. Gertrude Henle. Two antigens were used: (*a*) Frost 16th-passage amniotic fluid, dried from the frozen state and stored at —20 F. (the antigen was concentrated two and one-half times by reconstitution in a correspondingly smaller volume of distilled water) and (*b*) Chicago 12th-passage amniotic fluid, stored frozen at —70 C. The control antigen consisted of dried normal amniotic fluid, stored at —20 C. and concentrated two and one-half-fold at the time of reconstitution.

One-tenth milliliter of antigen and control material was injected in two sites in each forearm. Readings were made at 30 minutes and 24 hours, and a difference of 10 mm. or greater at the later interval between the average diameters of the antigen and control reactions was considered to be a positive test.

Volunteers.—All experiments were performed in healthy men between the ages of 21 and 28 years. A complete history and physical examination were obtained; hemoglobin and white blood cell count were measured, and urine was examined from each volunteer. Specific tests of liver function were then performed. All men with any abnormality, either in the general examination or in the laboratory tests, were excluded.

Liver-Function Tests.—The tests used and the normal values are as follows: serum bilirubin, < 1 mg. per 100 cc.[8]; sulfobromophthalein retention, 10% or less in 30 minutes[9]; thymol turbidity, < 7 units †[10]; thymol flocculation, < 2+ in 24 hours,[11] and cephalin-cholesterol flocculation, < 2+ in 24 hours.[12] The serum tests were performed at weekly intervals during the experiments, and, in addition, the semiquantitative urine urobilinogen determination[13] (normal value, < 1.5 Ehrlich units) was done approximately every other day. The Harrison spot test[14] for urine bilirubin was performed when indicated.

Hospital Facilities.—The prison hospital was arranged in such a manner that two 12-bed isolation wards were available. Each was equipped with a washroom and toilet. A resident physician and either one or two senior medical students were present at the institution throughout the entire experiment. Throughout the course of the experiments the only persons entering

† A ground glass standard was used, as prepared by Dr. Reinhold for the Armed Forces Epidemiological Board.

these wards were the physicians and the medical students. Food was prepared in the hospital kitchen and supplemented by ourselves when deemed necessary. All meals were served by the above named personnel, and precautions for intestinal isolation were observed. All articles leaving the wards were either autoclaved or boiled for 15 minutes. Each volunteer was seen and examined at least once daily. Temperatures were measured twice daily, and weight, at least once a week. In order to try to standardize results a list of possible symptoms and signs was prepared. This was checked daily, and comparisons were then made.

EXPERIMENTAL PROCEDURE AND RESULTS

Inoculation with Egg-Passage Materials Thought to Contain Hepatitis Virus.—Twenty-four healthy volunteers were inoculated orally with the following materials. Eight received 5 ml. of Frost 13th-passage whole egg emulsion; eight received 5 ml. of Chicago 12th-passage egg material, and eight received 5 ml. of a mixture of Frost 13th-passage and Chicago 12th-passage materials. This latter group received the skin tests described below on the same day as inoculation with the virus. Thirteen of the volunteers developed a mild illness without jaundice, characterized by minimal gastrointestinal symptoms, slight hepatic tenderness, and slight, but definite, hepatic enlargement. There were also mild abnormalities in the urine urobilinogen, sulfobromophthalein retention, serum bilirubin, and thymol turbidity tests. In addition, seven of the volunteers had very minimal departure from normal in one or two of the above symptoms, signs, or laboratory tests, but these were not considered conclusive enough to warrant classifying these men in the disease group. In Table 1 the various symptoms, signs, and abnormal liver-function tests observed in each volunteer are listed. Only the greatest deviation from normal function is listed. It can be seen that maximal abnormalities did not all occur at approximately the same time in the same or different persons, as is usually seen in infectious hepatitis. For this reason it is difficult to establish a definite incubation period either in the individual volunteer or for the group as a whole. However, the signs and symptoms with one exception developed after two weeks of incubation.

Results of Skin Test and Correlation with Disease Produced by Embryo-Adapted Virus.—Eight volunteers were tested with both skin test antigens. These were applied with the antigen above the control skin test on one forearm and below the control on the other forearm. Tests were read at 24 hours, as suggested by Drake and co-workers.‡ [7] As can be seen from Table 2, the skin test was positive to the Chicago antigen in three volunteers and to the Frost antigen in three others. It was negative to both antigens in two volunteers. It is of interest that all eight volunteers who received the skin tests were thought to develop disease after ingestion of the embryo-passage material.

Challenge of Volunteers with Natural (Unadapted) Virus After Previous Exposure to Egg-Adapted Virus.—The data presented above suggest that the egg-passage materials contained an agent producing an illness in volunteers, as previously reported.§ Although the clinical and laboratory observations were compatible with a very mild hepatitis, they did not furnish proof that the agent transmitted was IH virus. It was decided, therefore, to determine whether these persons had developed

‡ Dr. Jeremiah A. Barondess, of the Philadelphia Laboratory, helped in the application and interpretation of these tests.

§ References 2 and 3.

TABLE 1.—*Symptoms, Signs, and Results of Liver-Function Tests*

	Symptoms and Signs								Liver-Function Tests *						
Volunteer No.	T. over 100 F.	Anorexia	Nausea	Vomiting	Abdominal Pain	Weight Loss, Lb.	Hepatic Tenderness	Hepatomegaly	Bilirubin, Mg./100 Cc.	BSP, % Retention	Thymol Turbidity, Units	Thymol Flocculation	Cephalin Flocculation	Urine Urobilinogen, Ehrlich Units	Disease
1	+	+	+	0	+	5	+	0	0	15 D.17	0	0	0	4 D.24	+
2	0	+	+	0	+	6	+	0	0	0	7.7 D.38	0	0	1.6 D.15	+
3	0	+	+	0	0	0	+	+	0	0	0	0	0	3.2 D.15	+
4	0	0	0	0	+	4	+	0	1.1 D.25	+	0	0	0	1.7 D.40	+
6	0	+	+	0	+	0	+	+	1.3 D.51	13 D.42	0	0	0	4.33 D.41	+
13	+	+	0	0	0	6	+	0	0	0	6.2 D.18	0	0	1.55 D.21	+
14	+	+	+	0	+	0	+	+	0	14 D.38	0	0	0	3.7 D.22	+
15	0	+	+	+	+	9	+	+	1.3 D.38	0	0	0	0	2.20 D.39	+
16	+	+	+	+	+	0	+	0	1.4 D.38	0	0	0	0	2.15 D.23	+
17	0	+	+	+	+	3	+	+	0	20 D.18	0	0	0	2.25 D.25	+
18	+	+	+	+	+	0	+	+	1.1 D.38	11 D.38	0	0	0	0	+
23	0	0	0	0	0	0	+	+	0	23 D.18	0	0	0	2.18 D.24	+
24	0	0	0	0	0	0	0	+	1.5 D.38	20 D.18	5.6 D.18	0	0	2.25 D.39	+
8	0	0	0	0	0	8	±	+	0	11 D.39	0	0	0	0	±
9	0	0	0	0	0	0	±	+	0	11 D.39	0	0	0	1.80 D.29	±
10	0	0	0	0	0	0	0	0	1.1 D.9	13 D.18	0	0	0	1.60 D.15	±
12	0	+	+	0	+	0	+	0	0	0	6.2 D.17	0	0	1.9 D.17	±
19	0	0	0	0	0	9	0	+	0	28 D.18	0	0	0	1.87 D.11	±
20	0	+	±	+	0	0	+	0	0	16 D.18	0	0	0	2.03 D.21	±
21	0	0	0	0	0	0	0	+	0	11 D.11	0	0	0	2.60 D.39	±
5	0	0	0	0	0	0	±	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	2.08 D.15	0
11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	+	1.6 D.38	0	0	0	0	0	0

* D, day after inoculation; 0, no abnormality noted.

an immunity to unadapted virus. Thirteen of the previous volunteers and 8 control volunteers were selected. Sixteen weeks after the original ingestion of the chick-embryo-passage material all the persons were inoculated by the oral route with a total of 2.4 ml. of plasma obtained from volunteers during the early acute stage of illness

TABLE 2.—*Relation of Skin Sensitivity to Susceptibility to the Disease Observed Following Inoculation of Embryo-Passage Material in Eight Volunteers*

Volunteer No.	Antigen		Illness Produced by Embryo-Passage Material (Frost and Chicago)
	Frost	Chicago	
1	+	0	+
2	0	0	+
3	+	0	+
4	0	0	+
13	+	0	+
14	0	+	+
15	0	+	+
16	0	+	+

TABLE 3.—*Correlation Between the Disease Following Inoculation with Chick-Embryo-Adapted Virus and That Following Challenge with Natural Hepatitis Virus*

Patient No.	Experiment 1 (Egg-Passage Material)		Experiment 2 (Akiba Plasma)			
	Evaluation of Hepatitis	Virus Strain	Evaluation of Hepatitis	Jaundice	Skin Test	
					Frost	Chicago
Challenged patients						
2	+	Frost & Chicago	+	0	0	0
4	+	Frost & Chicago	+	0	0	0
14	+	Frost & Chicago	+	+	0	+
16	+	Frost & Chicago	+	+	0	+
17	+	Frost	+	0	..	..
19	±	Frost	+	+	..	..
12	±	Chicago	+	0	..	..
1	+	Frost & Chicago	0	..	+	0
13	+	Frost & Chicago	0	..	+	0
23	+	Frost	0	..	..	..
7	0	Chicago	0	..	..	..
11	0	Chicago	0	..	..	..
22	0	Frost	0	..	..	..
Controls						
25	..	.....	+	+	..	..
26	..	.....	+	+	..	..
27	..	.....	0	..	..	..
28	..	.....	0	..	..	..
29	..	.....	0	..	..	..
30	..	.....	0	..	..	..
31	..	.....	0	..	..	..
32	..	.....	0	..	..	..

induced by the unadapted Akiba strain. Plasma from eight different patients was given separately in 0.3 ml. amounts diluted in tomato juice at half-hour intervals. This procedure was employed because it was suspected that some of the plasmas contained antibodies, since preliminary attempts to titrate a pool of aliquots of these plasmas gave variable results (see under Materials and Methods). Table 3 correlates the results of this experiment with the experiment in which embryo-passage material

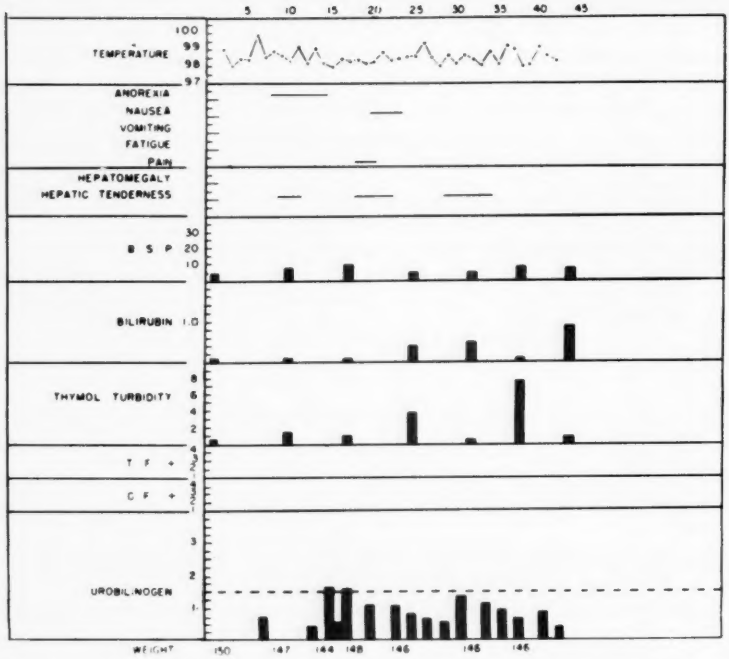

Fig. 1 (Volunteer 2).—Course of disease after inoculation with chick-embryo-passage material.

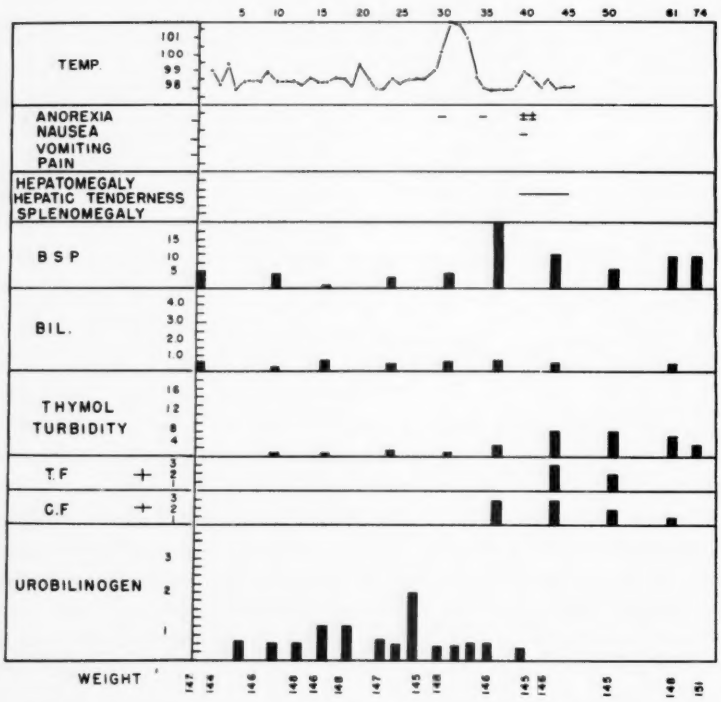

Fig. 2 (Volunteer 2).—Course of disease after inoculation with Akiba virus.

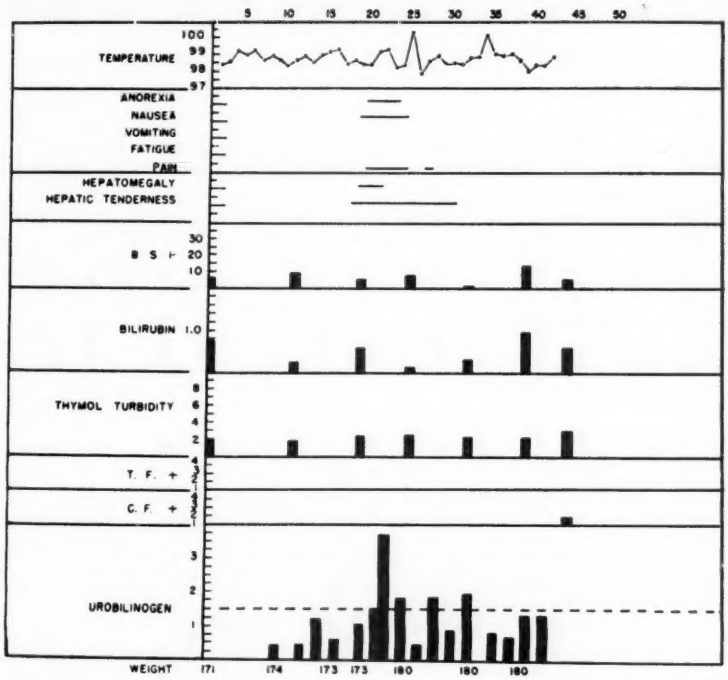

Fig. 3 (Volunteer 14).—Course of disease after inoculation with chick-embryo-passage material.

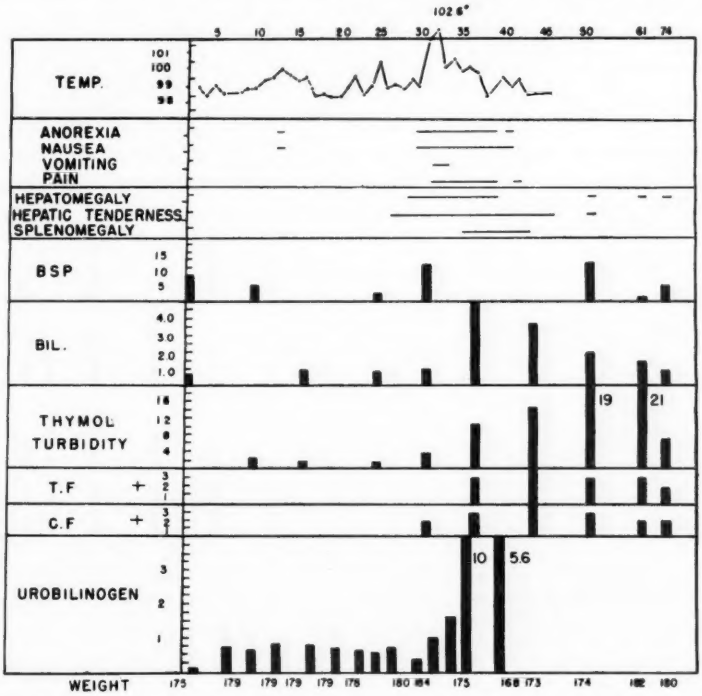

Fig. 4 (Volunteer 14).—Course of disease after inoculation with Akiba virus.

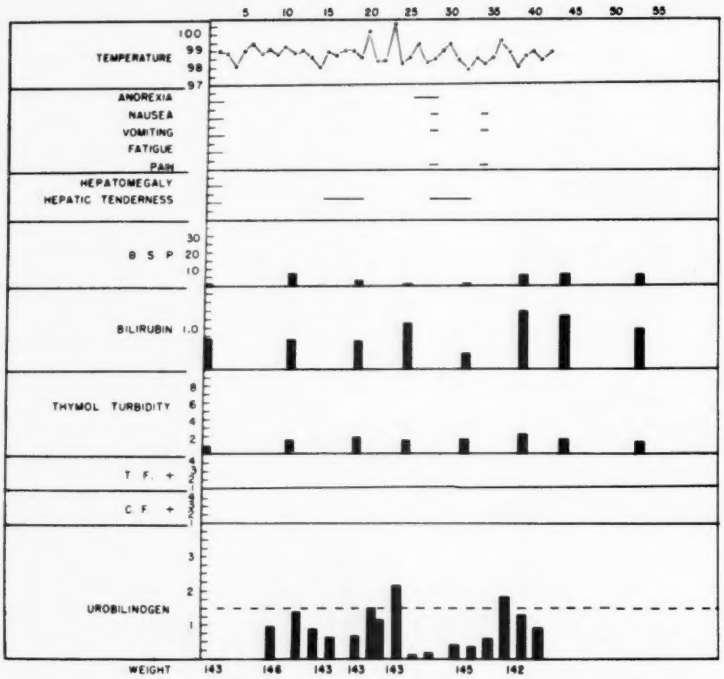

Fig. 5 (Volunteer 16).—Course of disease after inoculation with chick-embryo-passage virus.

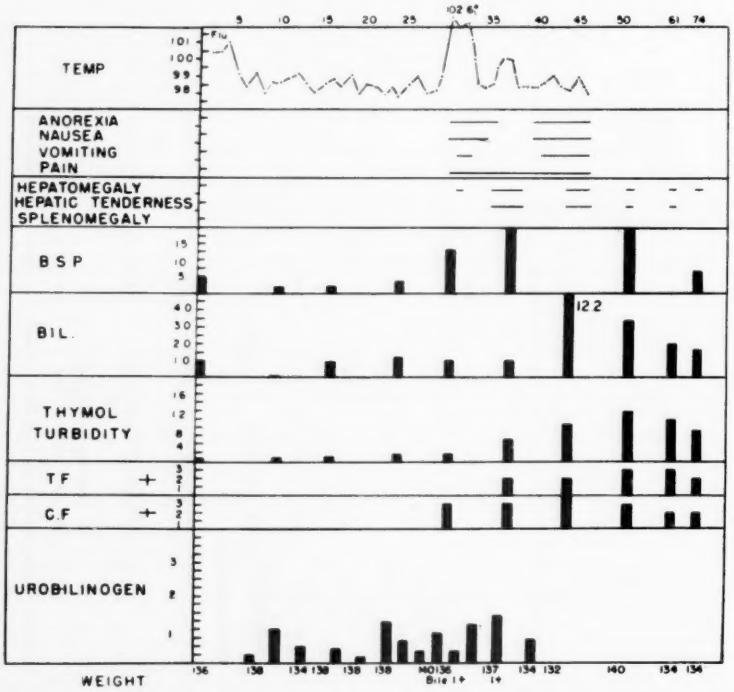

Fig. 6 (Volunteer 16).—Course of disease after inoculation with Akiba virus.

was given. Of the original volunteers, five who had suggestive evidence and two who had equivocal evidence of disease from the embryo-passage material developed hepatitis on challenge with the Akiba (IH) virus; three had jaundice, and four had unequivocal hepatitis without jaundice. Three volunteers who were thought to have been ill in the previous experiment remained well during the challenge experiment, and three developed no disease on inoculation with either the embryo-passage material or the plasma containing the Akiba strain of hepatitis (IH) virus. Two of the eight control volunteers developed hepatitis with jaundice. Figures 1 to 6 illustrate the typical disease observed following inoculation with chick-embryo-passage material and the subsequent hepatitis seen in three volunteers after inoculation with Akiba (IH) virus. Thus, no evidence of immunity was obtained. The somewhat lower incidence of hepatitis in the controls may be explained by the fact that these volunteers were Negroes, whereas all the others were white.

COMMENT

The data presented indicate that the inoculation of volunteers with egg-passage materials thought to contain hepatitis virus produced a mild illness with the characteristics recorded earlier. This disease might be considered to be mild hepatitis without jaundice, due to a hepatitis virus which had been attenuated by passage in the developing chick embryo. The disease differed from that which is usually observed in volunteers receiving unadapted hepatitis virus. The abnormalities in liver function observed in the illness produced by the embryo-passage material occurred over a period of approximately one month in the same person, with different tests being abnormal at different times. These abnormalities did not always correlate with the symptoms and signs. Although it was thus impossible to determine the incubation period accurately, it usually exceeded two weeks. This has not been the case in the disease produced by unadapted virus in the present study, where the person remained well, with normal liver function, for several weeks and then developed all the characteristic abnormalities, of clinical signs and symptoms and of liver function, at approximately the same time. However, in other volunteer experiments with unadapted virus the incubation periods were found to vary over a greater range, and mild cases comparable to those observed with the adapted virus were seen on occasion.[3] Because of these dissimilarities, the nature of the egg-adapted agent has not been identified. It seemed essential, therefore, to test for the development of immunity to hepatitis (IH) virus in the volunteers previously inoculated with embryo-passage material before assuming that the embryo-passage material was modified hepatitis (IH) virus. As shown in the preceding section, there was no apparent immunity.

There are several possible interpretations of the data presented.

1. There may have been no virus in the embryo-passage material. Against this is the fact that these volunteers did not remain completely well but apparently developed a disease as a result of oral inoculation with the chick embryo materials. Furthermore, the disease could be passed from one volunteer to others, as seen by the derivation of the Frost strain. In this respect one might consider "human factors" in the studies concerning (*a*) the observers and (*b*) the volunteers. With regard to the former, the clinical observers in the Philadelphia group have changed repeat-

edly over the nine years the studies were in progress, and now the disease induced was seen by an independent team. In general, the same findings were reported by all. As to the volunteers, it is obvious that they may exaggerate symptoms, but liver-function tests and hepatomegaly presumably are not subject to such influences.

2. There may have been some virus other than hepatitis virus present in the chick embryo material. Such hypothetical virus may have been "picked up" in the chick embryo. It is known that several animal species harbor hepatitis viruses of their own.‖ Such an agent derived from chick embryos may have caused the mild illness described. The observations of Bang [22] with the electron microscope suggest that an unidentified virus may exist in some normal embryos. However, if that were so, it would be difficult to explain why a disease with a relatively short incubation period is induced with material derived from an IH passage series, whereas a prolonged incubation period was seen after injection of an amniotic fluid of an SH passage series in chick embryos [23] or in chick embryo tissue culture.[24] The same objections would also hold for the suggestion that agents other than IH virus were "picked up" in the human material used for initiation of the passage series.

3. Hepatitis virus serologically unrelated to the challenge Akiba strain may have been present in the embryo material, which resulted in little or no cross immunity. The evidence thus far suggests that only one antigenic type of hepatitis (IH) virus exists. Although few experiments have been performed, Havens [25] and Neefe, Stokes, and Gellis [26] have demonstrated homologous immunity in studies done with unadapted virus. The results obtained by Henle and his associates [2] indicated resistance to a different strain (NL strain) of hepatitis (IH) virus in six volunteers who had received Akiba strain culture virus. Moreover, two of the cases of hepatitis occurred in the challenge group of volunteers who had received embryo-passage material of the Frost strain of virus and were thought to develop disease. As stated previously, this strain is probably identical with the Akiba strain used in the challenge experiment.

4. The amount of virus given as the challenging dose may have been so large as to overcome immunity. Even after convalescence from infection with natural virus immunity of volunteers to the homologous agent may not always be solid.¶ Since no titration of the virus used for challenge was done, this possibility cannot be denied.

5. The period of time between experiments may not have been optimal for the development of immunity. Although Henle and co-workers [2] waited six to eight months before challenging their volunteers, it is hard to imagine that if immunity were to develop it would not be present about 12 weeks after the onset of disease and 8 weeks after apparent recovery. However, the culture virus infection might have continued to smolder, as possibly indicated by the prolonged period of abnormal liver-function tests, and the challenge may have served to reactivate and aggravate the process.

6. Hepatitis virus may have been present but modified antigenically so that the disease produced by it did not confer immunity against unadapted hepatitis virus. The experience with chick-embryo-adapted measles virus may have a bearing on this situation, in that this agent became rapidly attenuated on passage and did not

‖ References 15-21.

¶ References 25 and 26.

induce solid immunity in susceptible children.# Mumps virus, likewise, rapidly lost its pathogenicity for man on passage in chick embryos.* Such attenuated virus sprayed into the oral cavity still evoked after three to five weeks the appearance of complement-fixing antibodies against the soluble antigen in a high percentage of children and against the virus antigen in a few. These results indicated an infectious process; however, neutralizing antibodies were rarely formed,[31] and one might presume, therefore, that it did not induce immunity with regularity.

7. Finally, one may raise the question whether full-blown hepatitis in man is the result of a dual infection, as in the case of hepatitis in mice described by Gledhill, Dick, and Andrews.[20] If that were so, one might speculate that only one of the agents has been propagated in the chick embryo and thus only a mild disease is induced. However, immunity to one presumably ought to protect against the development of severe disease caused by the combined infection.

It is impossible to say what the ultimate answer to the current dilemma may be. At present it is felt that the nature of the agent carried in chick embryo passages has not been proved to be IH virus, but, by the same token, its identity with IH virus has not been definitely excluded.

SUMMARY

Oral administration of chick embryo material presumably containing infectious hepatitis virus produced in volunteers a disease compatible with a very mild hepatitis. No evidence was obtained of the development in the volunteers of immunity to unadapted virus under the conditions of the experiment. Various possible explanations for these results are discussed.

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PERICARDIAL TAMPONADE

Diagnosis and Treatment

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ACUTE pericardial tamponade is not encountered frequently in a general medical practice, nor is it likely to become commonplace even in the more specialized experience of the cardiologist. Nevertheless, it is an important mechanical disorder, because failure to recognize it, or delay in initiating treatment, may mean death for the patient or progression of an underlying treatable disease.

During the past few years we have had the valuable experience of seeing and treating a number of patients with pericardial tamponade in a large hospital. As the cases in this series accumulated, three facts became apparent: 1. The diagnosis of pericardial tamponade is frequently missed. 2. There is need to stress the dangers of the acute tamponade. 3. Surgical approach is of great value in selected cases.

DESCRIPTION

Pericardial tamponade results when accumulation of fluid within the pericardial sac or constriction of the pericardium by scar or tumor tissue seriously interferes with the pumping action of the heart. It has been known for many years that the essential mechanical fault is restriction in diastolic filling when the pressure of fluid or constricting tissue prevents full expansion of the chambers during the resting phase of the cardiac cycle. A complex interrelationship of reduced cardiac output, altered venoauricular pressure gradients (from the encompassing fluid or scar), and the fluctuating intrathoracic pressure of respiration is productive of an important physical sign, called pulsus paradoxus. This term refers to fall in cardiac output during inspiration, the fall being measured with the blood pressure cuff or by palpation of the pulse. It is not actually a paradoxical effect but is an accentuation of the normal fall in output during inspiration.* The other important circulatory effect of acute tamponade is increased venous pressure, which frequently produces liver enlargement. Ankle edema and ascites are rarely seen.

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Read before the Section on Internal Medicine at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 23, 1954.

*An excellent summary of the mechanisms which produce this effect has appeared in a recent review article.[3]

DIAGNOSIS

In this series of 17 cases only two requirements were laid down for the selection of cases: 1. Proof of diagnosis must have been established by pericardial tap or thoracotomy. In the one case in which neither procedure was done (Case 12) the diagnosis of effusion was established by a characteristic sequence of x-ray films. 2. Every case must have been seen by the senior author. This requirement makes for a uniformity of experience not to be had in random collection from the record room.

In 11 of the 17 cases of acute tamponade the diagnosis was missed by the first physician to see the patient, and in a few instances it was missed by more than one examiner. The percentage of missed diagnoses was much higher than one would expect in the commoner acute clinical emergencies, such as congestive heart failure or appendicitis, and it seemed worth while to inquire into the reasons for this.

The symptomatology of this disorder appeared to be one source of difficulty. Dyspnea is practically always present in well-developed pericardial tamponade,

TABLE 1.—*Reasons for Missed Diagnosis* *

1.	Misconceptions preserved in the literature and transmitted as clinical lore
	Neck veins not distended
	Heart sounds too good
	Apex beat too strong
	Friction rub too loud
	No orthopnea
	Falling venous pressure (terminal)
	Insufficient rise in venous pressure
	Pulmonary second sound louder than aortic sound
	Ventricular pulsations on fluoroscopy too good
	Lack of "characteristic" change in heart shape on x-ray with change in position
2.	Missed physical signs
	Pulsating neck veins (5 cases)
	Pulsus paradoxus (9 cases)

* These are statements taken from the clinical records of patients with proved pericardial tamponade. They were intended as arguments against the diagnosis of tamponade, when it actually existed.

but, of course, this symptom does not of itself call the diagnosis to mind. Dyspnea is more likely to suggest congestive heart failure or pulmonary disease to the examiner. It was present in every case in our series and was always distressing in far-advanced tamponade. Cough was present in 5 of the 17 cases, but it is not particularly suggestive in itself. Pain was present in 9 of the 17 cases but varied in severity, quality, duration, and location, depending upon the underlying process responsible for the tamponade. When it was characteristic pericardial pain—substernal or precordial and varying with respiration—it helped to call attention to the possibility of pericarditis. However, in two cases in which pericarditis was diagnosed at once, developing tamponade was missed. In two cases pain was epigastric, presumably from distention of the liver.

Another source of difficulty lay in misconceptions about this syndrome existing in the mind of the examiner. These were apparently implanted by textbooks, transmitted to him by his medical teachers, or formulated on the basis of "common sense" deduction. Thus, in four cases the diagnosis of pericardial tamponade was seriously considered but was eventually rejected for reasons based on misconceptions. The reasons for rejection of the diagnosis as stated in the clinical records

are listed in Table 1, under the first heading. It will be seen at once that several of the stated reasons against the diagnosis are based on the assumption that fluid accumulation in the pericardium muffles the heart sounds, dampens the apex beat, and abolishes ventricular pulsations on fluoroscopy. While pericardial fluid may have this effect, it does not have it invariably. Two of our patients had excellent heart sounds. The first misconception listed (distended neck veins) is a favorite and will be taken up in detail under missed physical signs. Orthopnea is not necessarily present in this syndrome, and its absence sometimes helps to differentiate tamponade from congestive heart failure. It used to be thought that change in cardiac silhouette by fluoroscopy with change in position from lying to sitting was an important sign. However, when the pericardium becomes tense with fluid change in position may have very little effect on the shape of the heart shadow.

The third and most important source of difficulty in making the diagnosis of tamponade lay in failure to recognize important physical signs produced by rising venous pressure and falling cardiac output. Evidence of increased venous pressure on the basis of physical examination was missed in 6 of our 17 cases, and in two cases in which elevation was recorded by manometer its significance was misinterpreted. It is the belief of one of us, with special interest in the subject, that the proper examination of cervical veins for evidence of increased pressure is a neglected art. This is so partly because of misinformation on the subject to be found in medical textbooks. Unfortunately, there is a cherished misconception extant which stresses cervical vein distention as the *sine qua non* of elevated venous pressure. In the first place, many normal persons, particularly heavy-set ones, have considerable distention of neck veins when lying down, but this disappears in the upright position. Therefore, the subject must be seated or cranked up in bed to as near the vertical position as possible. Cervical vein distention in this position usually means very high venous pressure, such as that encountered in the upper mediastinal syndrome or in tricuspid disease. We did not see a single instance of distended neck veins in the sitting position in our 17 cases of pericardial tamponade. On the other hand, all but one had pulsating neck veins in that position. These are easily and frequently missed in routine examination unless certain simple principles are followed. Each side of the neck should be examined with the head rotated slightly to smooth out the skin. Then, the examining light should be shined across rather than at the neck, to highlight the pulsating vessels. A light shined at the veins will obscure their pulsations, and ordinary room lighting is often inadequate for visualization. The examination is best carried out with a flashlight in a darkened room. Pulsating neck veins are also seen in congestive heart failure, pulmonary embolism, and constrictive pericarditis, but they are rarely seen in the normal person sitting upright.

The consecutive frames from strips of movie film reproduced in Figure 1 illustrates the problem of neck vein signs. In the first series of frames, labeled *A*, taken from a patient with congestive heart failure, constant distention of the large neck vein can be seen in all pictures. This is the finding not present in our cases of pericardial tamponade. The second strip of consecutive frames shows the changing shadows produced by cervical vein pulsations in a patient with pericardial tamponade (Case 16). Both of these patients were seated, with a strong light shining across the neck.

While evidence of increased venous pressure is very important in the diagnosis of tamponade, it has less specific diagnostic significance than the misnamed physical sign "pulsus paradoxus." This important clue to diagnosis was overlooked by the first examiner in 9 of our 17 cases. Again, wide misconception about pulsus paradoxus exists, and many do not know that proper use of the sphygmomanometer is essential to early detection of the sign. First, the top systolic level is ascertained during normal breathing, and then the patient is requested to breathe deeply and regularly but not to the limit of inspiration or expiration. As this is going on, the column of mercury is allowed to fall slowly. In the normal person a drop of about 5 points in systolic pressure will be observed during inspiration. As pericardial

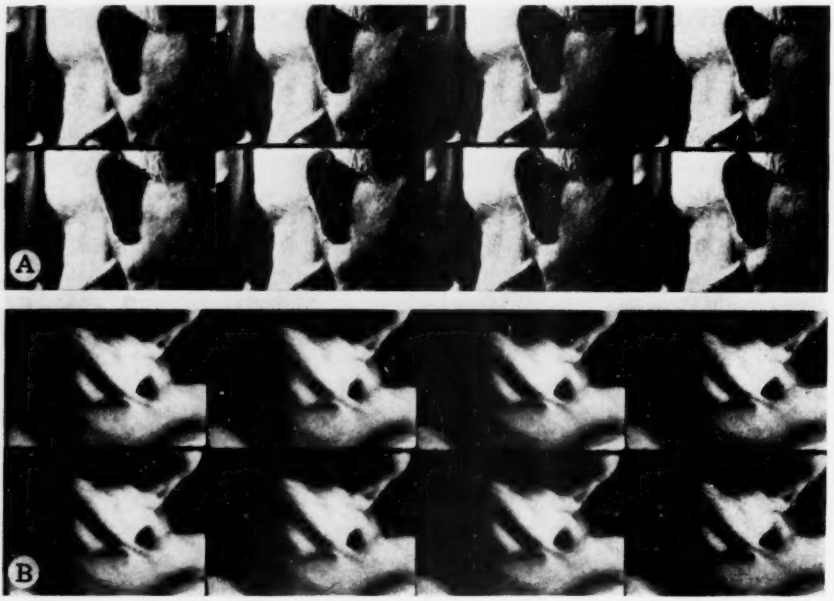

Fig. 1.—*A*, the constant degree of distention in a patient with congestive heart failure. *B*, close inspection shows a changing shadow just below and parallel to the ridge of the sternocleidomastoid. This is venous pulsation.

tamponade develops, the drop with inspiration becomes more pronounced, and with the syndrome well established it may not be possible to hear any sounds during inspiration. At this advanced stage of development the physical sign can be picked up by palpation of the radial pulse, but this cannot be relied upon in earlier stages.

If a patient is seen for the first time in the terminal stage of tamponade, the diagnosis may be very difficult to establish. As interference with circulation progresses, the patient goes into a state of collapse, characterized by disappearance of the arterial blood pressure and pooling of venous blood in viscera and extremities. Such a situation may be difficult to differentiate from myocardial infarction or

other disorders associated with shock. However, the degree of cyanosis in hands and feet resulting from venous pooling is out of proportion to cyanosis elsewhere in the body.

It must be remembered that pulsus paradoxus is encountered in other disease pictures, such as bronchial asthma, acute bronchiolitis from inhalation of irritating gases, pneumothorax, and congestive heart failure with peripheral vascular collapse, but seldom is the effect on circulation so pronounced as it is in advanced tamponade.

It was our experience that the diagnosis of tamponade was most likely to be missed by persons relying on book knowledge for differential diagnosis. When experience has been gained in the detection of physical signs, little difficulty is encountered.

TABLE 2.—*Summary of Etiologic Factors and Diagnostic and Therapeutic Procedures in Seventeen Cases of Pericardial Tamponade*

	Tap	Biopsy	"Window"
Tumor (7 cases)			
Undifferentiated carcinoma	X	X	X
Carcinoma, breast	X	X	X
Carcinoma, breast	X	X	X
Carcinoma, ovary	X	X	X
Oat-cell carcinoma	0	X	Attempted
Mesothelioma (?)	0	X	X
Thymoma	X	0	0
Infection (4 cases)			
Proved tuberculosis	X	X	0
Proved tuberculosis	X	X	0
Probable tuberculosis	X	0	0
Pneumococcus	X	0	0
Hemorrhage (1 case)	0	0	0
Nonspecific (4 cases)	X	X	X
	X	0	0
	X	X	X
	X	X	X
"Group" disease (1 case)			
Lupus (?)	X	0	0

CLINICAL PROCEDURE

Once the diagnosis has been made, the physician is faced with the decision of what to do about it and when to do it. The clinical course, x-ray findings, and electrocardiogram will help to differentiate between acute tamponade and the more chronic progression of constrictive pericarditis from scar or tumor growth.

In all of our cases but one (Case 12) some sort of definitive procedure was done, once the diagnosis of tamponade had been established with reasonable certainty. Pericardial tap was performed one or more times in 14 of the 17 cases. As might be expected, the more radical procedures of thoracotomy with biopsy and pericardotomy (pleuropericardial "window") were done less often (Table 2). The reasons behind the choice of these various procedures will be outlined under discussion of their specific etiology. Certain general principles governing pericardial tap should be mentioned at this time. Pericardial tap is done to establish the presence of fluid tamponade, to identify the etiologic agent if possible, and to provide relief of the tamponade, frequently a lifesaving procedure. The techniques of pericardial tap have been described by Kotte and McGuire.[1] At the Massachusetts

General Hospital, the xiphoid approach is preferred; the procedure is usually done under surgical conditions of asepsis, in the operating room. The xiphoid approach is both extrapleural and extraperitoneal, thus avoiding possible contamination of other cavities with infected pericardial fluid. Should bleeding or cardiac arrest occur, they can be handled more expeditiously in the operating room. Many studies of intracardiac and intravascular pressures have been made on humans and animals in the presence of pericardial tamponade. Studies on artificially produced tamponade in dogs, with saline solution introduced into the pericardial sac, show that the rise in venous pressure and fall in mean arterial pressure is rather slow until a

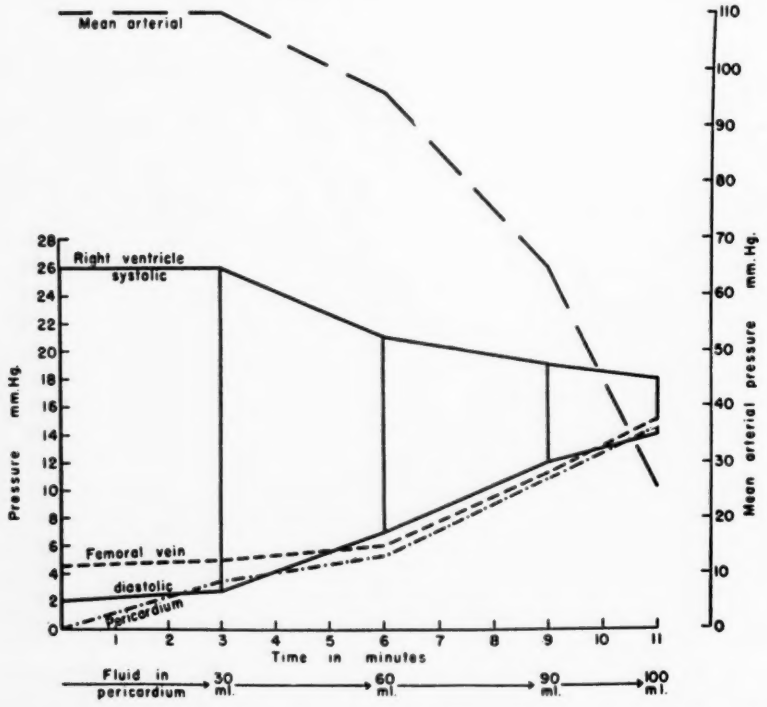

Fig. 2.—Typical circulatory changes produced by injecting fluid into the pericardium. From Evans, J. M.; Walter, C. W., and Hellems, H. K.: Alterations in the Circulation During Cardiac Tamponade Due to Pericardial Effusion: An Experimental Study Employing Cardiac Catheterization, *Am. Heart J.* **39**:181 (Feb.) 1950.

certain critical figure is reached (Fig. 2). Beyond that point, the drop in arterial pressure becomes very rapid. Conversely, the curves suggest that when arterial pressure has fallen to a dangerously low level in tamponade removal of only a few cubic centimeters of fluid will provide a considerable rise in pressure. Repeatedly we have seen dramatic improvement in clinical state following the removal of only a few cubic centimeters of fluid. This is of some practical significance, in that the removal of pericardial fluid may be difficult, no matter how great the intrapericardial pressure. It is sometimes useful to introduce air into the pericardial sac at the time

of tapping for the purposes of x-ray study. Occasionally it is possible to visualize the exact thickness of the pericardium by this maneuver and also to demonstrate residual fluid levels. However, introduction of air is not without hazard. One of our patients (Case 16) went into collapse after the introduction of air into the pericardial cavity but survived without other complications. No other untoward effects of pericardial tap were observed in any of our patients. The pericardial fluid may have characteristics of diagnostic value, but it should be remembered that the finding of bloody fluid does not limit the possibilities to tuberculosis or tumor. Patients with so-called "nonspecific" pericarditis may have bloody fluid. Fluid is especially valuable for diagnosis when it is purulent, as in our one case of pneumococcal pericarditis (Case 11). Further comment on the value of pericardial fluid in diagnosis will be found under the discussion of specific etiologic groups. In many patients, one pericardial tap may be sufficient to provide a diagnosis and to relieve the situation of tamponade permanently. However, fluid accumulates rapidly when the stimulus to formation is strong, as in tumor, tuberculosis, and occasionally in "nonspecific" pericarditis. It is then necessary to reach a decision as to whether further taps should be relied upon to keep the patient alive or whether a more extensive procedure such as biopsy or pleuropericardial "window" production is indicated.

PLEUROPERICARDIAL "WINDOW"—TECHNICAL CONSIDERATIONS

Although direct drainage of the pericardium is the best method of treating suppurative pericarditis, it is not suitable for prolonged use when the pericardial fluid is sterile, because of the danger of infection. The establishment of a large opening between the pericardium and pleural cavities will give immediate and sustained relief of tamponade. Accumulated fluid in the pleural cavity can then be drained with little hazard of causing suppurative pericarditis.

Most of the patients who have been treated by "pleuropericardial windows" have been desperate surgical risks because of severe tamponade. To avoid operative deaths, the following precautions have been taken. First, general anesthesia has been employed only when there was a reasonable certainty that intubation in the presence of cardiac failure and anoxia could be tolerated. Otherwise, the procedure has been carried out with local procaine anesthesia and oxygen administered under pressure through a face mask. So far no difficulty has been experienced in maintaining the expansion of the lung by this method. Second, the patients have usually been placed on their backs in a semiupright position during operation, to permit the full use of both lungs.

The operative site has been selected according to where the maximum prominence of the heart was seen on the chest roentgenogram. As a rule, an incision has been made between the third and fourth or the fourth and fifth ribs on the left, starting at the sternum and running laterally for about 6 in. (15 cm.). To provide adequate exposure, the adjacent costal cartilages have been cut close to the sternum. After ligation and division of the mammary vessels, the ribs have been gently separated. In the presence of either malignant changes or infection the identification of pleura, pericardium, and myocardium as separate structures can be very difficult. The introduction of a No. 19 needle (with a short bevel) for aspiration of fluid has been helpful in establishing the thickness of the layers covering the heart. A

bloody effusion is thinner, more purple, and under less pressure than ventricular blood. Traction sutures have been placed in the pericardium occasionally to create a space between it and the myocardium. The initial incision has usually been small, so that the release of pericardial pressure would be gradual.

The "window" itself must be as large and as low as possible. The overlying lung must be retracted so that it cannot seal over the opening. Accordingly, a portion of pleura and pericardium measuring about an inch (2.5 cm.) in diameter has been resected from over the apex of the heart. The anteromedial edge of the overlying lung has then been sutured to the posteroinferior edge of the opening with interrupted 00000 silk sutures, to form a pleural dead space outside of the window. A No. 24 Foley catheter with a 30 cc. bag has been placed in this artificial space close to, but not touching, the heart. Its end has been brought out between the ribs below the incision (Fig. 3). After closure of the operative wound

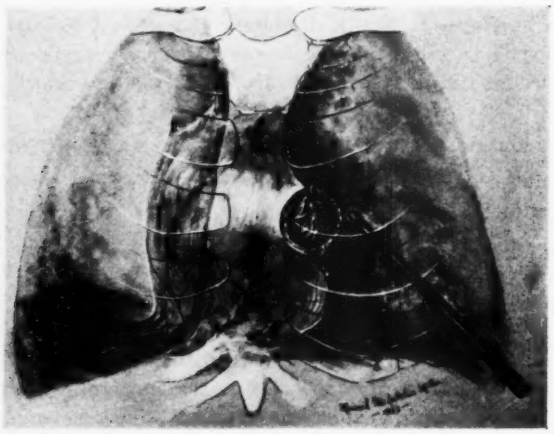

Fig. 3.—Schematic drawing showing pleuropericardial "window," with the lung sutured at the outer margin and chest catheter in place.

in the routine manner, suction with a constant negative pressure of about 8 cc. of water has been applied to the catheter until fluid has ceased to accumulate. Usually four or five days of drainage have been necessary, during which time the patient has been protected by antibiotics. The intrapleural cavities so formed have gradually filled in, without evidence of infection.

CLASSIFICATION OF CAUSES OF PERICARDIAL TAMPONADE

Tumors.—Study of Table 2 shows that the greatest single cause of pericardial tamponade in our series was tumor. As might be expected, the greatest number of thoracotomies was done in this group. In the one patient with thymoma (Case 7) the diagnosis had been established several years previously by thoracotomy. Invasion of surrounding lung tissue was so great that no further surgical procedure was considered feasible. However, a pericardial tap did provide temporary relief. In the other six patients with tumor, pericardial biopsy was done. Successful

"window" procedures were done in five of these. In one patient with oat-cell carcinoma (Case 5) an attempt was made to produce a "window" but tumor invasion was too extensive and tenacious. In the patient with questionable mesothelioma (Case 6) a mesothelioma of the lung had been removed nine months before. At operation no evidence of tumor was found, and biopsy showed only acute and chronic pericarditis. Therefore, this finding was quite reassuring, and adequate relief was provided by the window. The patient with metastatic adenocarcinoma had had a cyst of the ovary removed 10 years before her first pericardial "window" operation was performed (Case 4). When the pericardial biopsy showed metastatic adenocarcinoma, presumably from the ovary, the patient was treated with radiation, with excellent results. However, she returned after a period of seven months with troublesome tamponade, and another pericardial fenestration was done. Two more fenestration procedures were necessary in the ensuing three months, and at the time of the last operation seeding on the pericardial and ventricular surfaces was evident. The patient obtained considerable relief from symptoms by the procedures, but enough tumor remained to produce both pulsus paradoxus and cervical venous pulsation. One patient with carcinoma of the breast (Case 2) was benefited by pericardial "window" and lived for one year. The second patient with carcinoma of the breast (Case 3) had at least two and one-half years of active life after the window was made and x-ray therapy given. The patient with extensive undifferentiated carcinoma (Case 1) died soon after operation. Thus, it was felt that at least four out of seven patients with tumor as the etiologic factor profited from surgical treatment, in that the symptoms of pericardial tamponade were relieved and the obtaining of tissue from the pericardium made intelligent x-ray therapy possible. In five of the seven tumor cases, cytological examination of the pericardial fluid was done. Three of the five were reported as positive; two, negative.

Infection.—Of the three cases labeled tuberculosis, two were proved and one was probable. In one of the proved cases (Case 8) biopsy, culture of the pericardial fluid, and guinea pig inoculation were all positive for tuberculosis. However, in this patient—an indigent woman—it was possible to make a diagnosis by biopsy approximately three months before it could have been made by the more routine methods. Although other evidence of tuberculosis, such as chest lesions and positive sputum specimens was not present, it was possible to get this patient admitted to a sanitarium for chemotherapy on the positive evidence of pericardial biopsy. She has had an excellent result, with arrest of her tuberculosis and complete recovery of health. In the other instance of tuberculosis proved by biopsy (Case 9) the diagnosis was suspected because of continued high fever and prostration but was opposed by some of the physicians who saw the patient, on the grounds of laboratory findings; that is, sugar level in the pericardial fluid was normal; concentrated fluid was negative for tubercle organisms on smear, and there was no evidence of tuberculosis in roentgenograms of the chest. With the evidence of positive biopsy, it was possible to proceed with chemotherapy for tuberculosis. The patient made an excellent response to treatment. Later, inoculation of a guinea pig was positive for tuberculosis. In the patient with "probable" tuberculosis (Case 10) the diagnosis was qualified. Evidence of active infection was obtained by smear from the sputum, but culture of the pericardial fluid was negative for tuberculosis

and the guinea pig died of other cause before the characteristic lesions could have developed. Under the circumstances, biopsy was considered unnecessary, and evidence of pericardial effusion disappeared with chemotherapy. Six months after tap, the patient was doing well, with nothing to suggest active tuberculosis.

In our only case of purulent pericardial effusion (Case 11) produced by infection with Pneumococcus, the true diagnosis was unsuspected until tamponade developed, and pericardial tap was made necessary by collapse of the patient. The diagnosis of purulent pericarditis was then obvious. Unfortunately, diagnosis in this patient was delayed by postponement of pericardial tap, which should have been done three days earlier for relief of tamponade. In retrospect, the chief errors here lay in failure to recognize tamponade, then in failure to act promptly when it was recognized. Falling venous pressure was taken as a sign of recovery, when it actually presaged collapse. Immediate relief was provided by tapping the pericardium, but death followed within a few hours from rupture of the aorta through a metastatic abscess in its wall.

Hemorrhage.—No instance of hemorrhage due to trauma was encountered. However, a patient with extensive anterior myocardial infarction (Case 12) developed pericardial tamponade in the second week of his illness. The onset was characterized by rather vague substernal and left shoulder pain worse on breathing; increasing dyspnea and liver enlargement suggested congestive heart failure, but impressive paradoxical pulsation pointed the way to the correct diagnosis. Venous pulsation and distention were absent. A series of chest x-rays showed typical increase in heart shadow without pulmonary congestion, followed by return to normal. Also, this patient had two characteristics described by Goldstein and co-workers[2]: high temperature and leucocytosis (leucocyte count of 30,000). While hemorrhagic effusion is probably commoner in patients who receive anticoagulant therapy for myocardial infarction, it also occurs as a complication of infarction in patients not receiving such therapy.† No pericardial tap was done in our patient because he improved when the use of bishydroxycoumarin (Dicumarol) was stopped. Also, it was felt that pericardial tap in his case might be particularly hazardous because of the danger of penetrating the large necrotic area of myocardium with a needle. However, it was felt that all evidence pointed to hemorrhagic tamponade complicating myocardial infarction. In this patient, the pulsus paradoxus was overlooked for the first two days of onset of this complication because nurses' blood pressure readings were relied upon. Only when the physician in charge took the blood pressure himself was it realized that pulsus paradoxus was present. Also, it is very important to remember that high temperature and leucocytosis are apt to accompany this complication. This fact does not seem to be generally realized.

"Nonspecific Pericarditis."—In this group there were four cases. Nonspecific pericarditis is essentially a diagnosis of exclusion, and there is still some argument as to whether this condition is of infectious or allergic nature.[3] This group of cases is usually characterized by temperature rise, acute onset of pericardial pain, and friction rub. How many of these cases actually represent tuberculous pericarditis

† Myers, G. S.: Personal communication to the authors.

has not been established, since it is well known that fluid from tuberculous pericarditis may be negative on culture and guinea pig inoculation. Three of our cases had thoracotomy with pericardial biopsy and the pleuropericardial "window" procedure. This seems like a high incidence of such procedures in so-called nonspecific pericarditis, but it must be remembered that all these cases had troublesome pericardial tamponade recurrent after tapping and that the diagnosis was very much in doubt. Therefore, it was felt justified to relieve the tamponade by pleuropericardial "window" procedure and to get tissue for analysis at the same time. In each instance the tissue slides showed nonspecific changes of pericarditis, with no evidence of tuberculosis or tumor involvement. Nevertheless, judgment must be reserved on these cases, since by chance some of the areas selected for biopsy could have been outside the areas of active tuberculous involvement. However, all

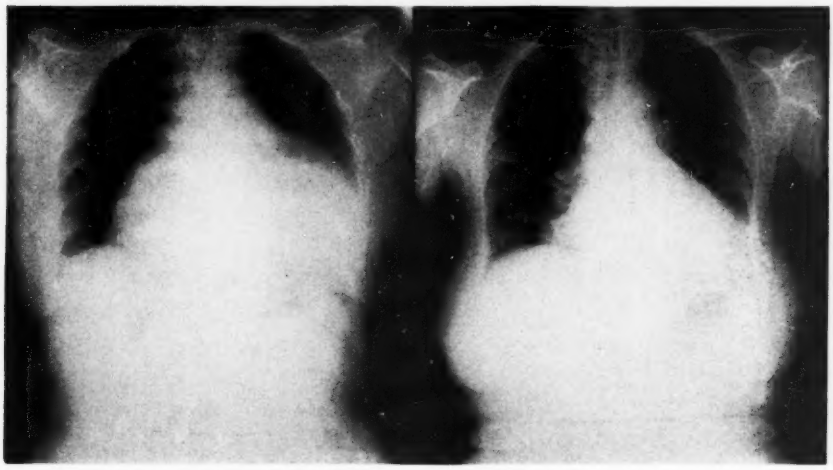

Fig. 4.—Pericardial effusion subsiding. See text for details.

the patients have done well. Roentgenograms taken in Case 16 (nonspecific pericarditis) are shown in Figure 4. The first film, taken Nov. 25, 1953, shows the massive pericardial effusion before operation. The second film was taken March 11, 1954, after the patient had been up and about at home. The residual enlargement is at least partly on a basis of hypertensive heart disease. She is known to have had considerable enlargement before the onset of pericardial effusion.

"Group" Disease.—In Case 17 the diagnosis of pericardial tamponade was proved by tap. However, it was not felt that thoracotomy was indicated. Therefore, no definite diagnosis was made, since the patient's fluid culture was negative for tuberculosis. She was thought to have one of the "group" diseases, possibly lupus erythematosus. While pericarditis is relatively common in lupus and other "group" diseases, particularly rheumatoid arthritis, pericardial effusion of large size is most unusual, and we have not seen pericardial tamponade in rheumatoid arthritis or lupus erythematosus. Therefore, the diagnosis in this patient is also in doubt.

TABLE 3.—*Case Summaries*

		Reasons Given Against Diagnosis	Missed Physical Signs*	Symptoms	Pericardial Taps	Pericardial Fluid Studies†			Pericardial Window	Pericardial Biopsy	Results and Remarks	Diagnosis
						Tumor Cells	Culture	Guinea Pig				
Tumor												
1	G. S.	.....	.....	Pain, dyspnea	1	+	Neg.	Died of intercurrent infection	Yes	Undifferentiated carcinoma	Died 3 days after operation	Undifferentiated carcinoma
2	I. W.	.....	.....	Dyspnea	2	+	−	−	Yes	Metastatic carcinoma	Good immediate results; died 1 yr. after operation	Carcinoma of breast
3	R. B.	Called heart failure	P. P. P. N. V.	Dyspnea	1	+	....	....	Yes	Metastatic carcinoma	2½ years of active life after window + x-ray therapy; died 3 yr. after window	Carcinoma of breast
4	R. O.	.....	.....	Dyspnea, cough, abdominal fullness	3	Neg., done on first tap only	Neg.	ND	Yes, 4 times	Metastatic carcinoma	Recurrent tamponade; comfortable years after 1st window, 1 mo. after 2d	Carcinoma of ovary
5	A. McL.	Called heart failure	P. P.	Dyspnea, cough, dysphagia	0	ND	ND	ND	Attempted, impossible technically	Oat-cell carcinoma	Died after operation	Oat-cell carcinoma of lung
6	A. M.	Good pulsations, cardiac shadow by fluoroscopy	P. N. V. P. P.	Dyspnea, pain	0	Neg.	ND	ND	Yes	Acute and chronic pericarditis	Good immediate results; no follow-up; mesothelioma of lung removed 9 mo. before	Mesothelioma (?); could be nonspecific pericarditis
7	J. G.	.....	.....	Dyspnea, cough, pain	1	ND	ND	ND	No	....	Died years after thoracotomy for removal thymoma	Thymoma
Infection												
8	S. B.	.....	P. P.	Dyspnea, epigastric pain	1	ND	+ Tbc	+	No	+ for Tbc	Excellent results from chemotherapy	Proved tuberculous pericarditis

9	L. C.	Pulsus paradoxus noticed but not given enough weight	.....	Dyspnea	1	ND	ND	+	No	+ for Tbc	Excellent results from chemotherapy	Proved tuberculous pericarditis
10	P. R.	No distension of neck veins; paradoxical effect too little (not true); venous pressure readings not high enough (120-140)	P. P. P. N. V.	Dyspnea, cough	1	ND	Neg. for Tbc	Died of intercurrent infection	No	No	Did well on chemotherapy	Probable tuberculous pericarditis
11	M. S.	Failing venous pressure (actually approaching collapse); significance of pulsus paradoxus not realized	.....	Dyspnea	1	ND	+ for pneumococcus	ND	No	No	Died of ruptured aortic abscess	Pneumococcal pericarditis
Hemorrhage												
12	M. S.	P. P. missed because of reliance on nurses' blood pressure readings	P. P.	Pain, dyspnea	0	—	—	—	No	No	Did well after bishydroxycoumarin was stopped and vitamin K was started	Myocardial infarction with hemorrhagic effusion
Nonspecific												
13	E. G.	Good apex beat; heart sounds and friction rub too loud; no rise in venous pressure	P. P.	Dyspnea	1	ND	Neg.	Neg.	Yes	Chronic inflammation	No recurrence	Nonspecific pericarditis
14	M. M.	.....	.....	Dyspnea, pain	3	ND	Staphylococcus albus	Neg.	No	No	Good recovery	Nonspecific pericarditis
15	R. M.	Significance of elevated venous pressure not realized (pressure 260 cm. of water)	P. P. P. N. V.	Pain	1	Neg.	Neg. for Tbc	Neg.	Yes	Acute and chronic fibrinopericarditis	Good recovery	Nonspecific pericarditis
16	S. R.	Heart sounds too good	P. N. V.	Dyspnea, pain	1	Unsatisfactory	ND, smear neg. for AFB	ND	Yes	Chronic inflammation	Excellent recovery; seen 4 mo. after operation	Nonspecific pericarditis
Group Disease												
17	R. R.	No venous distension	P. P. P. N. V.	Pain, dyspnea (?)	1	ND	Neg. for Tbc	ND	No	No	Good recovery	Lupus erythematosus (?)

* P. P., pulsus paradoxus; P. N. V., pulsating neck veins.
† ND, not done; Tbc, tuberculosis; AFB, acid-fast bacilli.

SUMMARY

Seventeen cases of pericardial tamponade are reviewed. The diagnosis was frequently missed owing to misconceptions about the syndrome of tamponade and because of failure to recognize the important physical signs—pulsus paradoxus and pulsating neck veins. Dangers of acute tamponade are stressed. The procedures of pericardial tap and biopsy are discussed, and a section is devoted to the description of pleuropericardial "window." The use of all these procedures in diagnosis and treatment is outlined.

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ACUTE HEPATIC INSUFFICIENCY OF THE CHRONIC ALCOHOLIC

Clinical and Pathological Study

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MOST CHRONIC alcoholics with active but uncomplicated cirrhosis demonstrate rapid improvement in their liver disease, in our experience, following hospitalization and consumption of an adequate diet. At times, however, acute severe hepatic functional impairment occurs, which may clear slowly or progress until the patient dies in hepatic coma. In this situation, the liver may show, in addition to fat and fibrosis, an extensive lesion characterized by hyaline degeneration and necrosis of parenchymal cells with leucocytic infiltration. This lesion, which was described by Mallory in 1911 [1] and which occurs in varying degrees of severity in many alcoholics, is so distinctive that it was decided to determine further its clinical significance. To do this, the present study was carried out, in which the clinical and pathological findings relating to the liver in 56 chronic alcoholics were analyzed. It was found possible to divide these cases, according to histological findings, into two groups: Group I, those with a moderate amount or more of this lesion, and Group II, those with a slight amount or less. Other histological abnormalities, such as fat and fibrosis, were also evaluated.

MATERIALS AND METHODS

Clinical and pathological observations on 56 chronic alcoholics who had been on the wards of the Boston City Hospital were examined. All except two were thought to have been drinking to within a few days prior to hospital admission. None with known serious infection or hemorrhage just prior to or at the time of admission to the hospital were included.

The cases chosen for study were as follows: 13 patients with marked jaundice who had been observed clinically, of whom 10 died (9 with typical spontaneous hepatic coma), and who had had adequate pathological study (autopsy, 10; needle liver biopsy, 2; surgical liver biopsy, 1); 11 cases drawn from the autopsy files, 10 because of hepatic histological lesions similar to those

This investigation was sponsored in part by the Commission on Liver Disease, Armed Forces Epidemiological Board, with support by grants from the Office of the Surgeon General, United States Army, Washington, D. C.

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seen in the first group, and 32 patients who had had an autopsy (2 patients) or adequate needle liver biopsy (30 patients) and liver-function tests within four days after admission to the hospital. The liver specimens of these 32 patients were obtained on the day of admission in 21, on the second day in 5, on the third day in 5, and on the fourth day in 1; serial biopsies were performed in 12. Four of these patients died.

Various liver-function tests were performed. The methods for measuring the concentration of serum bilirubin, urinary urobilinogen, and serum alkaline phosphatase have been referred to in a previous report.[2] The icteric index was done according to Ham.[3] When both serum bilirubin concentration and icteric index were measured in the same patient, the former determination, converted to icteric index by multiplying by a factor of 10, was used. The concentration of urinary bile was estimated either by the "foam test" or by the Harrison spot test.[4] The serum prothrombin concentration was measured by the method of Quick,[5] and the cephalin-cholesterol flocculation test was done as described by Hanger.[6]

PATHOLOGICAL METHODS AND CRITERIA

Needle biopsies of the right lobe of the liver were performed with the Vim-Silverman needle, usually by the intercostal approach. For serial liver biopsies, an attempt was made to insert the needle close to the site of the previous puncture. The specimens were fixed in 10% formalin and stained with hematoxylin and eosin. Surgical biopsies were fixed in Zenker's fluid with acetic acid and stained with phloxine and methylene blue. Autopsy material, generally obtained from the right hepatic lobe, was fixed in Zenker's fluid with acetic acid, 10% formalin, and in some instances Zenker's fluid with formalin (Helly's fluid) and were usually stained with phloxine and methylene blue. Phloxine and hematoxylin and sudanophilic stains (Sudan IV and scarlet red) were used occasionally.

The following morphological features were preferentially evaluated and roughly quantitated:

1. Hyaline degeneration ("alcoholic hyalin"). This cytoplasmic change was described by Mallory in 1911[1] and appears as irregular, frequently perinuclear clumps of an homogeneous highly refractile dense material. It is well demonstrated after fixation with Zenker's fluid with acetic acid and staining with phloxine and methylene blue and appears a deep rose-red in sharp contrast to the basophilic or amphophilic cytoplasm. With formalin fixation followed by hematoxylin and eosin staining, the hyalin stains a lavender color. Other methods of fixation and staining have been described.*

The hyaline change has only been demonstrated in hepatic cells. It may involve isolated cells or groups of cells or may be quite generalized, tends to be located in cells at the periphery of the lobule, and may be seen in cells showing sudanophilic vacuolization. The frequency with which liver cells exhibiting the hyalin are necrotic suggests that it is a cytoplasmic change indicative of serious cell injury. It has been considered in this laboratory as a frequent finding in the cirrhosis of alcoholics. Only rarely has it been observed in the cirrhosis of nonalcoholics or in other diseases and then only in minimal degree, and it differs in histological appearance from the hyaline necrosis seen in viral hepatitis and yellow fever.

2. Necrosis. The orthodox criteria for the histological identification of necrosis were used, i. e., pyknosis, karyorrhexis, karyolysis, and polymorphonuclear leucocytic infiltration.

3. Parenchymal disorganization. This refers to a disruption in lobular architecture in which the liver cells no longer maintain an orderly arrangement.

4. Fat. This was identified as unstained cytoplasmic vacuoles except in the few instances in which specific stains were used.

5. Fibrosis. New fibrous tissue was not differentiated from condensation of preexisting stroma.

6. Bile pigment. The presence and degree of ductal, canalicular, and parenchymal bile pigment was noted. The differentiation of parenchymal bile pigment from hemosiderin and lipofuscin was not made.

* References 1 and 7-9.

The degree of each of these histological changes when present was estimated on a 1+ to 4+ scale, a method at best semiquantitative; 1+ signified a slight degree; 2+, a moderate degree; 3+, a rather marked degree, and 4+, the most pronounced degree of change.

RESULTS

Histological examination of biopsy or autopsy liver specimens permitted the division of 54 of the 56 cases into two distinct groups: Group I, 28 cases with a

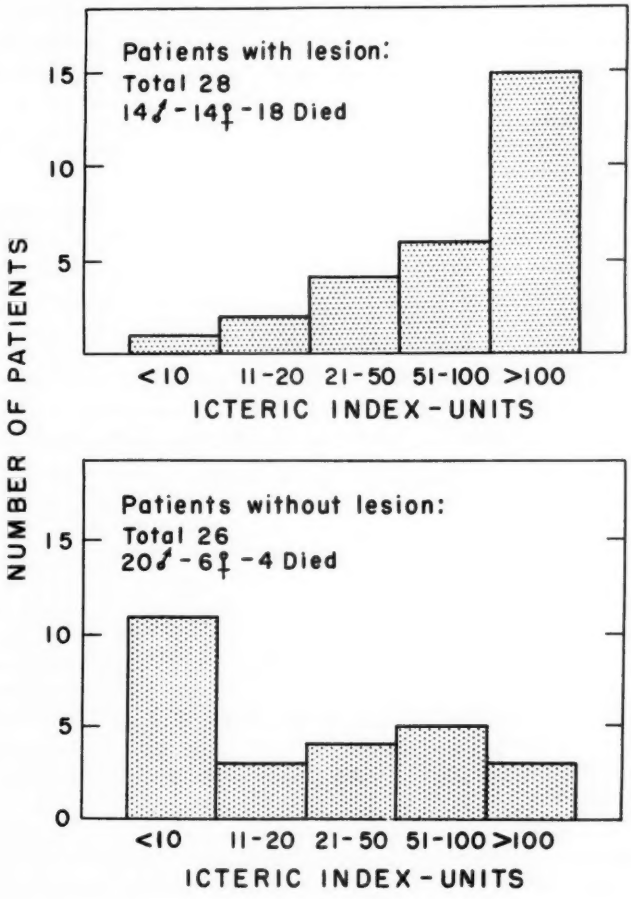

Fig. 1.—Icteric index.

moderate amount (2+) or greater of hyaline degeneration, liver cell necrosis, and parenchymal disorganization; Group II, 26 cases with a slight amount (1+) or less of each of these three elements. Two cases only were intermediate between these two groups and will not be considered further.

Because 54 of the 56 patients fell distinctly into either of these two groups, further analysis was made on this basis. Hereafter, the cases in Group I will be

referred to as those showing the lesion complex † and those in Group II as being without the lesion complex.

In Group I there were 14 men and 14 women, while in Group II there were 20 men and 6 women. When the icteric index of the patients in each group was examined, it was found that in Group I it was over 50 in 21 patients and over 100 in 15, while in the second group it was over 50 in only 8 (Fig. 1 and Table 2). Eighteen patients in Group I died; 5 in Group II died.

The 54 patients comprising these two groups were further analyzed both clinically and pathologically in the following groups: (1) the 18 patients in Group I (with the lesion complex) who died, (2) the 10 patients in Group I (with the lesion

TABLE 1.—*Data on Seventeen Patients with Lesion Who Died*

Historical data	
Age, yr.	
Range	30-63
Av.	Men, 47; women, 43
Sex	7 M; 10 F
Race	16 w., 1 Negro
History of chronic alcoholism	17
History of previous jaundice	4
History of poor diet	17
Physical examination	
Undernutrition	8/13 *
Jaundice on admission	16/17
Hepatomegaly on admission	16/17
Ascites on admission	11/17
Splenomegaly	8/17
"Spider" telangiectases	9/16
Temp. > 102 F. in hospital	6/17
Laboratory data	
Urine bile present	16/17
Hematocrit level less than 40%	10/14
Guaiac-positive stools	10/16
Cephalin-cholesterol flocculation (48 hr.) > 2+	11/14
Alkaline phosphatase > 5 B. U.	0/5
Prothrombin concentration < 30%	9/16
Blood non-protein nitrogen > 50 mg./100 ml.	5/11

* First number refers to number of patients with stated abnormality, and second number refers to number of patients in whom abnormality was commented upon.

complex) who lived, and (3) the 26 patients in Group II (without the lesion complex).

1. *Patients in Group I (with the Lesion Complex) Who Died.*—Although 18 patients fell into this category, the clinical and pathological data will be considered collectively only for the 17 patients who were autopsied. The other patient had two serial hepatic biopsies.

Historical Data (Table 1): Seven of the patients were men, and 10 were women. Sixteen were white, and 1 was a Negro. Their ages ranged from 30 to 63 years, with an average age of 44.5 years. The average ages of men and women were 47 and 43 years, respectively.

† The term "lesion complex" is used to denote an hepatic histological picture characterized by two degrees (moderate amount) or greater each of hyaline degeneration, liver cell necrosis, and parenchymal disorganization.

Few significant previous illnesses were uncovered. Four of the patients described previous episodes of jaundice, which had occurred from two to six years prior to this admission. Three had had delirium tremens; one, Korsakoff's syndrome; one, Wernicke's syndrome, and one, mental deterioration. Acute pancreatitis had occurred in one patient, and thyrotoxicosis, in two.

All the patients were known to be chronic alcoholics, drinking up to the time of hospital admission, except one, who admitted to chronic alcoholism for 40 years but denied imbibition during the six months prior to hospital entry. Several patients revealed an increase in aloholic intake for weeks to months before entry. They consumed whiskey, beer, or wine singly or in combination.

All the patients described a poor dietary intake for from months to years. Fourteen patients stated that anorexia became more severe in the few weeks to months prior to admission, with a consequent decrease in food intake, 9 patients eating almost nothing for from two to five weeks before entry. Four patients admitted nausea and vomiting for several weeks before admission.

Jaundice had been noted by only 10 patients for from two days to four months, with an average of 36 days, before entry. Only two patients had observed light-colored stools, and one, dark urine prior to entry.

Physical Examination (Table 1): Eight patients were considered poorly nourished or emaciated; four, fairly well nourished, and one, well nourished; in four, no comment was made concerning nutritional status. Peripheral neuropathy was observed in four, red tongue in two, cheilosis in one, and spongy gums in one. No other evidence suggesting nutritional deficiency was noted.

Every patient but one (the Negro) was jaundiced on admission. The jaundice was noted to deepen in five patients during hospitalization.

The liver edge was palpable on admission in 16 patients. Ascites prevented adequate palpation in the 17th. The size was not specified in 2, but in the remaining 14 it ranged from 2 to 9 fingerbreadths below the right costal margin, with an average of about 5 fingerbreadths. In five patients the liver was said to be tender. A decrease in the size of the liver during hospitalization was observed in four patients; in one of these it was 3 fingerbreadths below the right costal margin on admission and became nonpalpable during 34 days of hospitalization.

The spleen was palpated in eight patients and extended as far as 3 fingerbreadths below the left costal margin. The presence of ascites prevented satisfactory palpation in some of the nine patients in whom the spleen was not felt.

Nine patients had many prominent "spider" telangiectases, and six, palmar erythema.

Ascites was present in 11 patients on admission, questionable in 3, and absent in 3. One patient who had no ascites on admission developed it in the hospital.

Ankle edema was marked in 2 patients, slight to moderate in 3, equivocal in 1, and absent in 11.

The highest body temperature (usually oral) reached by each patient, which occurred on an average of 3.6 days after admission, was between 98.0 and 105.9 F.; it was 100.0 F. or less in 6 and 102 F. or less in 11.

Laboratory Data (Table 1): Bile was present in the urine in all patients but one, the only patient without jaundice. Urinary urobilinogen was measured in 16 patients. In 11 patients it ranged from a dilution of 1:2 to 1:512, four having a dilution of 1:256 or higher. In four patients, one of whom received chlortetracycline

(Aureomycin) intravenously, the urinary urobilinogen was absent, and in one it dropped from 1:256 to 0. Of these five patients in whom urinary urobilinogen was not demonstrated, three simultaneously had brown stools and another had a stool positive for urobilinogen, indicating that biliary obstruction was not complete.

Sixteen patients had stool examinations, with an average of between three and four specimens per patient. Nine patients had clay-colored or yellow stools during hospitalization; in six, all stools observed were of this color. Six patients never had a positive stool guaiac test; five had negative tests on admission which became positive; one had slightly positive tests throughout hospitalization, and four had all positive tests (in two of these never more than moderately positive).

Polymorphonuclear leucocytosis was a prominent finding and usually increased during hospitalization (Table 2).

The hematocrit level was determined in 14 patients, in 12 on the day of admission and in 2 one day later. In four patients it was over 40%; in six, between 30 and 40%; in three, between 20 and 30%, and in one, 19%. Eleven patients had a repeat hematocrit determination; in eight this was within three days of death. Three developed a significant decrease (between 7.5 and 17.5%); an increase occurred in five. Three patients had massive bleeding; in two this occurred one day before death, and in one, four days before death. Transfusions were given to these three, and in two the hematocrit level dropped less than 3%.

The icteric index (or serum bilirubin concentration converted to icteric index) was elevated in 16 patients on admission and remained the same or increased during hospitalization (Table 2). In one patient, who had two measurements made during hospitalization, both values were normal. This was the only patient in whom no jaundice or bilirubinuria was noted.

Seven patients had measurements of serum bilirubin concentration, which were repeated for two. The last determination of total serum bilirubin concentration done ranged from 15.5 to 37 mg. per 100 ml., with an average of 24.7 mg.

Cephalin-cholesterol flocculation tests (48-hour) in 14 patients were read as 4+ in 7, 3+ in 4, 2+ in 2, and 1+ in 1 (an average was taken when the test was repeated).

The serum alkaline phosphatase concentration was normal in the five patients for whom it was determined.

Plasma prothrombin concentration was measured in 16 patients. When an average value is used for each of the seven patients who had more than one determination, the range was 10 to 84%, with an average of 35.4%. In 9 of the 16 it was less than 30%. In one patient who had repeated determinations there was a downward trend.

The blood nonprotein nitrogen concentration, which was determined within 15 days of admission in 11 patients and within 2 days of admission in 8, ranged from 20 to 66 mg. per 100 ml., with an average of 33 mg. Subsequent measurements in 10 patients (in 8 within one day of death) ranged from 31 to 143 mg. per 100 ml., with an average of 60 mg. An average rise of 30 mg. per 100 ml. occurred in seven of the eight patients with determinations near the time of admission and death. Oliguria was a frequently observed phenomenon.

Course: The duration of hospitalization ranged from 1 to 34 days, with an average of 11.7 days. Eliminating the three longest durations, the average was 7.3 days. Eleven patients lived 10 days or less after hospitalization.

TABLE 2.—*White Blood Cell Counts and Icteric Indices of Patients With and Without Lesion*

Patients with Lesion Who Died										Patients with Lesion Who Lived					Patients Without Lesion				
		WBC Count					Icteric Index*												
		Initial¶		Final			Final					Initial† WBC Count		Initial ‡ Icteric Index,* Units			Initial§ WBC Count		Initial‖ Icteric Index,* Units
Patient	Days in Hosp.	WBC/ Cu. Mm.	% Polys	Days Before Death	WBC/ Cu. Mm.	% Polys	Initial‡ Units	Days Before Death	Units	Patient	Days in Hosp.	WBC/ Cu. Mm.	% Polys		Patient	Days in Hosp.	WBC/ Cu. Mm.	% Polys	
A. S.	6	6,600	77	2	16,100	93	>100	..	...	H. M.	53	7,500	59	135	J. D.	59	12,600	72	110
A. M.	17	7,500	73	0	24,400	86	100	0	>100	A. W.	90	18,900	48	120	T. G.	5	3,200	65	115
L. S.	2	9,100	71	..	.....	..	>100	1	155	H. S.	122	26,000	78	>100	A. M.	8	10,150	60	>100
K. R.	5	24,300	72	3	27,800	94	75	3	>100	P. S.	16	4,000	72	60	E. B.	22	10,400	68	70
E. T.	8	22,100	90	2	28,800	94	>100	1	>100	M. H.	32	4,850	61	75	C. M.	3	8,700	72	75
J. H.	15	19,500	64	3	30,600	..	200	3	220	J. T.	8	9,700	66	30	M. P.	20	14,000	..	75
A. R.	28	7,300	80	2	24,800	..	260	..	...	R. W.	19	12,200	81	25	F. C.	40	4,200	..	90
L. V.	5	15,400	91	2	31,000	82	>100	2	370	P. F.	36	21,800	84	20	H. C.	36	4,900	..	95
E. G.	6	10,900	77	..	.....	..	100	..	...	J. C.	10	7,400	61	11	A. L.	18	7,550	64	45
J. M.	2	13,900	80	..	.....	..	75	..	...	E. T.	5	11,000	77	20	B. R.	42	6,200	..	25
J. C.	10	7,700	79	2	23,800	..	90	3	180						J. Mc	43	5,700	69	30
A. Mc.	5	29,000	..	4	17,200	89	>10	4	>50						G. R.	1	7,100	..	35
C. D.	34	14,900	93	7	18,500	95	110	21	155						R. H.	15	5,700	72	15
E. G.	29	20,400	74	1	24,800	79	5	10	5						D. D.	30	14,000	69	18
W. F.	6	8,600	76	..	.....	..	30	0	220						T. M.	124	5,400	66	19
V. W.	1	4,600	60	..	.....	..	35	..	...						P. C.	15	6,500	63	4
L. B.	4	"Normal"	..	..	.....	..	320	..	...						H. O.	4	9,900	68	5
P. D.	26	16,700	87	14	30,500	87	170	10	175						F. S.	3	7,500	79	9
															J. M.	39	6,200	..	6
															T. D.	5	5,600	77	4
															J. Ma.	2	5,000	71	2
															J. D.	5	8,900	62	6
															H. W.	6	9,200	60	5
															H. D.	20	14,700	84	7
															J. McG.	7	7,600	78	8
															D. Da.	44	4,850	74	6

* In cases in which the serum bilirubin was determined it was converted to icteric index by multiplying by 10.[3]
† Done on day of admission in all cases.
‡ Values were determined as close to day of biopsy as possible, within one day of admission in 8 cases.
§ Done on day of admission in 24 cases.
‖ Values were determined close to day of biopsy as possible, within one day of admission in 21 cases.
¶ Done on day of admission in 14 cases.
‡ Done within one day of admission in 14 cases.

Eight patients were given a high-protein high-carbohydrate diet on admission, but most patients ate little, even the approximate amount being indeterminable. In the other patients, the type of diet was not specified. Multiple vitamins were administered in 11 patients. Eight patients received fluids parenterally, often dextrose intravenously being the only source of calories for days prior to death. Four patients were given penicillin; one, chlortetracycline, and one oxytetracycline (Terramycin).

Aside from melena (see above), the complicating illnesses included delirium tremens in three patients and generalized dermatitis herpetiformis in the one patient without jaundice. One patient developed a sort throat from which was cultured hemolytic Staphylococcus aureus; following oxytetracycline therapy protracted diarrhea ensued, also yielding hemolytic Staph. aureus, and this condition continued until death although the oxytetracycline was discontinued. Abdominal paracenteses were performed once in three patients and twice in one patient.

Nine patients were thought to be mentally clear on admission; seven were mentally sluggish, drowsy, or confused, and in one the mental state was not specified. Thirteen patients became progressively drowsier, lapsed into coma, and died over the course of a few days. The three patients with brisk gastrointestinal hemorrhage became comatose and died more quickly. One patient suddenly became agitated and cyanotic, had a convulsion, and died the day after entry. A "flapping" tremor [10] was observed in six patients, and the typical electroencephalographic changes of hepatic coma [11] were seen in the two patients on whom tracings were done. Hepatic fetor was noted in two patients, and urinary leucine crystals, in another.

Pathology ‡: Gross. All the livers in this group of 17 autopsy cases were conspicuously abnormal grossly. They were typically yellow or yellow-green and had irregular surfaces, which were granular; one liver showed coarse focal scarring. They were firm and cut with more resistance than normal. All but one (1,600 gm.) of the livers were enlarged, two weighing between 1,800 and 2,000 gm.; five, between 2,000 and 2,500 gm., and nine, over 2,500 gm. No evidence of extrahepatic biliary obstruction was found. Focal bile stasis, as evidenced by spotty yellow-green coloration of the liver, was noted in 10. Ascites was described in 12 patients; splenomegaly (spleen greater than 200 gm.), in 15, and esophageal varices, in 8. Recent massive upper gastrointestinal tract hemorrhage had occurred in five cases. Acute interstitial pancreatitis was observed in four cases, one of which showed abscess formation and fibrosis. The kidneys were moderately to markedly enlarged in nine cases (combined weight, between 400 and 560 gm.), slightly enlarged in four (340 to 380 gm.), and within normal limits in four (220 to 320 gm.). The kidneys were described as being bile-stained in 13 cases. In consistency, they were usually flabby, and they presented widened frequently pale cortices.

Microscopic (Figs. 2 to 7). The liver histology was remarkably similar in all 17 patients. Few liver cells were normal in appearance. Hyaline degeneration was outstanding and usually involved large groups of cells. At times a globule of hyalin appeared to represent the only remnant of the cell. Many of the liver cells showing hyaline degeneration presented irregular hyperchromatic nuclei and prominent nucleoli. The hepatic cells were often considerably frayed, with sometimes only cellular fragments remaining. Many cells showed swelling, with granularity and decrease of cytoplasmic staining. Liver cell necrosis was conspicuous, and cellular

‡ Drs. Frederic Parker Jr., G. Kenneth Mallory, and Dante F. Campagna-Pinto helped in the analysis and interpretation of the pathological material.

infiltration, especially of polymorphonuclear leucocytes, was usually prominent. Frequently, degenerated and necrotic cells were invaded or surrounded by the leucocytes. The degree of infiltration varied from slight to marked and in a few cases was striking in that in large areas of the parenchyma great numbers of leucocytes were seen. In most of the cases increased numbers of polymorphonuclear leucocytes were also seen in the sinusoids and blood vessels. Parenchymal disorganization was a prominent feature. Any semblance of lobular structure was often difficult to discern, and the tissue sections sometimes appeared as though they were artifactually macerated. Variation in size of cells and nuclei was common. Liver cells with multiple nuclei were frequently seen, but mitoses were rare.

Fibrosis was advanced in degree in every liver but one, in which it was thought to be slight. The fibrosis tended to be portal in distribution, with prongs of fibrous tissue extending to other portal spaces and into the lobules, with resulting disruption

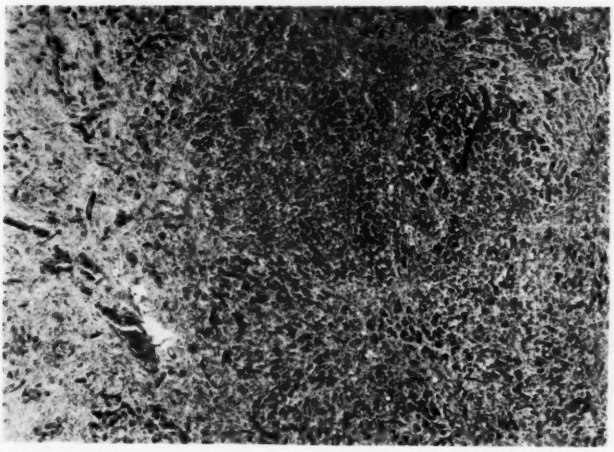

Fig. 2 (Patient A. R., Group I).—Disruption of lobular architecture is conspicuous, and the amount of parenchyma appears to be decreased. Note the minimal amount of fatty vacuolization present. Fibrosis is marked in degree and diffusely distributed. Phloxine and methylene blue; × 35.

of the lobular architecture. In addition, there appeared to be, in a few cases, a diffuse fibrosis involving most of the lobule. This was most apparent in those cases with extensive necrosis and polymorphonuclear leucocytic infiltration. Estimation of the extent of fibroblastic proliferation was difficult.

Intracellular bile accumulation as well as canalicular bile plugging was often outstanding. Ductal bile was rarely seen.

With respect to fat content, however, the histological picture differed impressively among these patients. Twelve exhibited a large amount of hepatic vacuolization, but in 4 it was only slight in amount, and in 1 none was apparent.

In the case of one of the patients who died in hepatic coma with this lesion complex autopsy was not done but two hepatic biopsies were made. The first specimen was obtained 3 days after admission, and the second, 22 days later, which was one day before death. The liver biopsies disclosed a severe lesion complex similar to

that seen in the 17 patients, with little apparent difference between the two specimens. Of particular note, however, was the considerable diminution of fat in the second biopsy specimen as compared with the first.

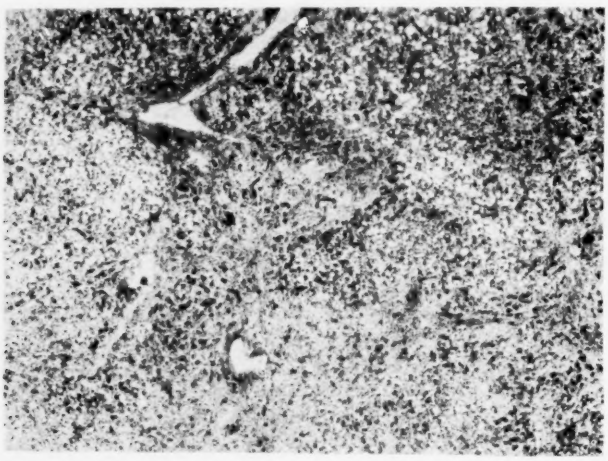

Fig. 3 (Patient E. G., Group I).—Parenchymal disorganization is so pronounced that little semblance of lobular architecture remains. The number of parenchymal cells appears to be considerably diminished. Fatty vacuolization is extensive, and fibrosis is moderate in amount. Phloxine and methylene blue; × 35.

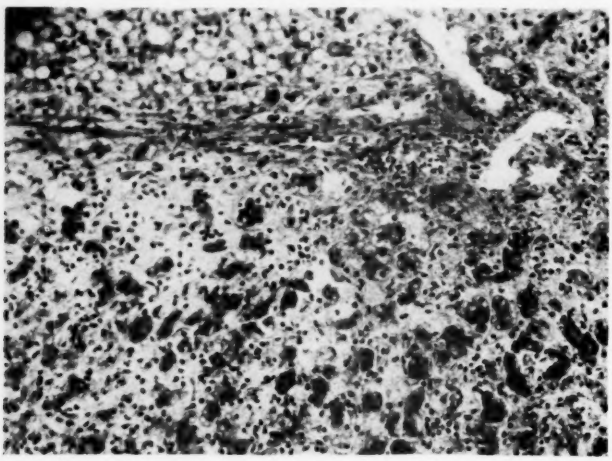

Fig. 4 (Patient E. G., Group I).—A higher power view of Figure 3, revealing hyaline degeneration and extensive cellular infiltration in addition to the parenchymal disorganization, fatty vacuolization, and fibrosis. Phloxine and methylene blue; × 140.

2. *Patients in Group I (with the Lesion Complex) Who Lived.*—Ten patients with a moderate amount (2+) or greater of hyaline degeneration, liver cell necrosis, and parenchymal disorganization by liver biopsy survived. One of these was readmitted about three years later with marked jaundice and died; at autopsy he showed

a severe hepatic lesion complex, and he was included in the previous group. Another of these was readmitted six months later with jaundice and also died, but no autopsy was performed. Two patients in this group had associated Wernicke's syndrome.

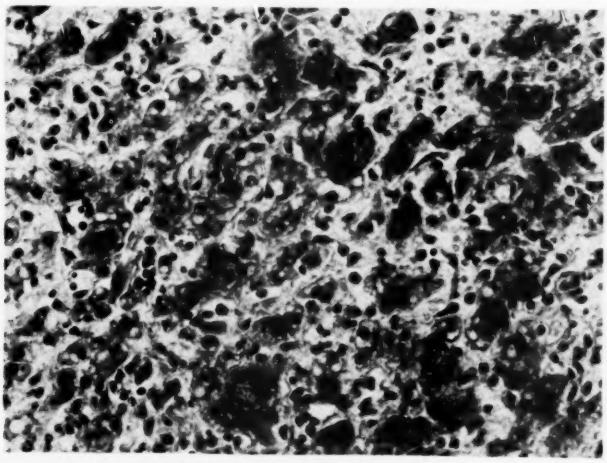

Fig. 5 (Patient E. G., Group I).—A higher power view of Figure 4, showing considerable hyaline degeneration, liver cell necrosis, parenchymal disorganization, and infiltration with leucocytes predominantly of the polymorphonuclear variety. Fatty vacuolization is also evident. Phloxine and methylene blue; × 240.

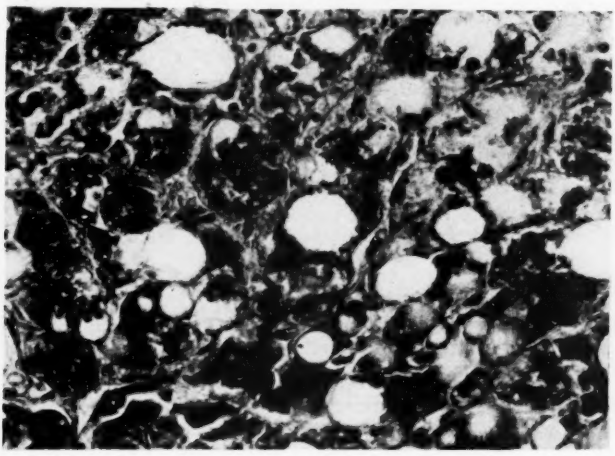

Fig. 6 (Patient V. W., Group I).—There is considerable fraying of the hepatic cells, with sometimes only cellular fragments remaining. Many swollen granular cells can be seen. Hyaline degeneration, liver cell necrosis, and fatty vacuolization are evident. Phloxine and methylene blue; × 305.

Five of the patients had a decidedly more severe lesion complex than the other five. Of the first five, three had icteric indices over 100 and one, between 51 and 100. In the fifth patient the icteric index was between 11 and 20, but this patient insisted

that he drank no alcohol and had increased his dietary intake for two weeks prior to entry because of the onset of jaundice and weakness. Thus, his icteric index may have declined during these two weeks. Of the five patients with a lesser degree of the lesion complex, the icteric index was between 51 and 100 in only one, and it was less than 50 in the other four.

The five patients with a lesser degree of the lesion complex and with less marked functional impairment improved rapidly. In contrast was the slowness with which the patients with a marked degree of the lesion complex and greater functional derangement improved, both clinically and histologically. Improvement was so slow in three of these patients that the diagnosis of extrahepatic biliary obstruction was seriously considered, and in one of these three an exploratory laparotomy was performed. Serial biopsies in another revealed a very gradual resolution of the lesion complex over the course of 50 days. A fourth patient with a marked lesion complex

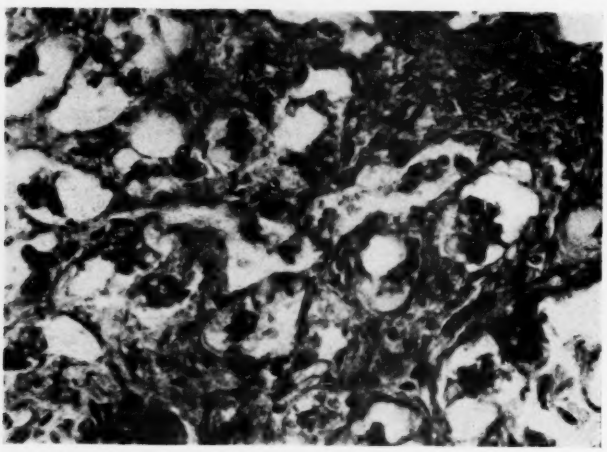

Fig. 7 (Patient L. V., Group I).—Many cells show swelling, with granularity and decrease of cytoplasmic staining. Some cell borders appear disrupted. Hyaline degeneration, liver cell necrosis, fatty vacuolization, and fibrosis are present. Phloxine and methylene blue; × 305.

had three liver biopsies, on admission and after 10 and 20 days of hospitalization, respectively. Although over this three-week period his total serum bilirubin dropped from 13.6 to 2.7 mg. per 100 ml. of serum, the three biopsies showed little change except for a progressive diminution of fat. The fifth patient with a severe lesion complex left the hospital against advice after five days, and his condition could not be evaluated subsequently.

All biopsy specimens of the patients in this group had moderate to marked amounts of hepatic fat, except for two in which it was slight. One of these two patients, as mentioned above, had changed his regimen two weeks prior to admission, and in the other biopsy was not done until after two to three weeks of hospitalization. The small quantity of fat in the biopsy specimens of these two patients, therefore, may have been related to the change in regimen prior to the biopsy. Moderate to marked degrees of fibrosis were evident in the biopsy specimens of all the

patients but one. No fibrosis was evident in the initial specimen from this patient, but two subsequent biopsies did show moderate amounts of connective tissue which was young in appearance, suggesting that little or no fibrosis was present on admission. It is possible, however, that the small initial biopsy missed the fibrosis.

3. *Patients in Group II (Without the Lesion Complex).*—Twenty-six patients fell into this category, in which there was histologically a slight amount (1+) or less of hyaline degeneration, liver cell necrosis, and parenchymal disorganization. Twenty were men, and six were women. In eight the icteric index was over 50, in three, over 100. Four of the six women had icteric indices over 50.

Five patients in this group died, and, although four had icteric indices over 50, none of the five was thought to have died in spontaneous hepatic coma. One of these, for whom a biopsy was done on admission, developed gastrointestinal bleeding with increased jaundice and died five days after entry with severe delirium tremens; no

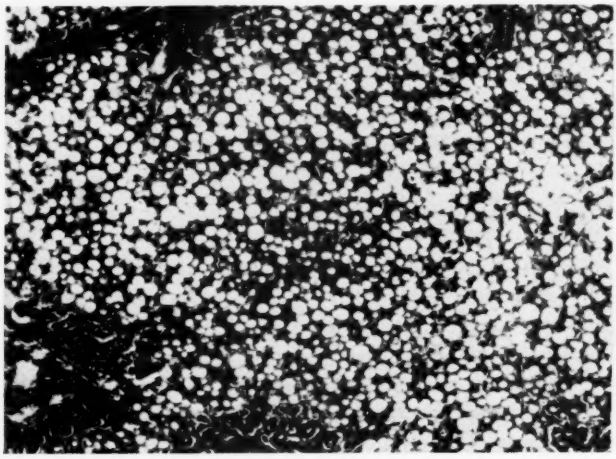

Fig. 8 (Patient A. M., Group II).—A large quantity of fat and a moderate amount of fibrosis are present. The lesion was not evident, even though this patient had an icteric index over 100 and died in coma after a dose of paraldehyde. Phloxine and methylene blue; × 47.

autopsy was obtained. Another patient developed coma rapidly after a 15 ml. dose of paraldehyde and died shortly thereafter. The third patient was thought to have an extrahepatic biliary obstruction, and an exploratory laparotomy was performed, but 10 days after operation, although the jaundice appeared to be diminishing, the patient went into shock and died. Another patient died with severe delirium tremens three days after entry. The fifth patient, whose icteric index was less than 50, died suddenly of unknown cause on the day of admission. Histologically, the livers of these four patients showed large quantities of fat and moderate amounts of fibrosis (Fig. 8).

The other four patients with icteric indices over 50 improved rapidly. The liver biopsy in two of these patients showed large quantities of fat and moderate fibrosis, but in the other two the fat and fibrosis were small in amount. In fact, in one of these the hepatic biopsy obtained on admission showed only occasional fat globules,

minimal fibrosis, probable rare hyaline degeneration, but much intracellular bile. Indeed, there was little apparent abnormality to account for the marked functional derangement.

The remaining 17 patients, who had icteric indices less than 50, generally had uneventful hospitalizations, with rapid improvement in the abnormal liver-function tests. The degree of hepatic fat and fibrosis varied considerably. Several patients had hepatic fat without fibrosis, but the reverse was not found, perhaps because all these patients were drinking up to the time of hospital admission. Three patients with marked amounts of hepatic fat but without fibrosis had no elevation of the icteric index.

COMMENT

From the present analysis it is evident that chronic alcoholics may show a characteristic hepatic lesion complex consisting of hyaline degeneration, liver cell necrosis, and parenchymal disorganization. Varying degrees of this lesion complex were seen to occur, and its three histological components, i. e., hyaline degeneration, liver cell necrosis, and parenchymal disorganization, appeared to parallel each other in intensity, so that when one was present in more than moderate degree so usually were the other two. These hepatic abnormalities have been reported several times § since the original description by Mallory in 1911,[1] but the lack of emphasis of this lesion complex may lead one to conclude that it is an unusual entity. On the contrary, our experience indicates that this lesion complex in some degree occurs frequently in hospitalized chronic alcoholics with liver disease who have been drinking until the time of admission to the hospital.

When the lesion complex was severe, it was characteristically associated with marked hepatic functional impairment, which sometimes eventuated in typical spontaneous hepatic coma and death. The extent of the parenchymal involvement left little reason to wonder why these patients developed hepatic failure. Hepatomegaly was constant, and usually present were splenomegaly, ascites, and spider telangiectasia. The most obvious evidence of the disturbance in hepatic function was bilirubinemia with jaundice, which was usually conspicuous and sometimes was accompanied by acholic stools. Of the 18 patients with the lesion complex who died, the icteric index was over 100 in 13 and over 50 in 16. In only one patient was it said to be normal. Considerable elevation in the icteric index can occur in alcoholics, however, in the absence of this lesion complex. Eight such patients were observed, in three the icteric index being over 100 and in eight, over 50. It would appear, therefore, that patients with a severe degree of the hepatic lesion complex almost constantly have jaundice, while chronic alcoholics with marked jaundice may or may not show the lesion complex, but usually do.

Another conspicuous accompaniment of the lesion complex was a polymorphonuclear leucocytosis, which usually increased as the patient grew worse. There was generally a pronounced decrease in plasma prothrombin concentration. The cephalin-cholesterol flocculation test was usually markedly positive, but it was sometimes normal, and the serum alkaline phosphatase was normal in the five patients in whom it was measured. Gastrointestinal bleeding occurred commonly. Several patients developed oliguria with rising serum nonprotein nitrogen concentrations terminally.

§ References 12-17.

Although cirrhosis appears to be much commoner in males,‖ 11 of the 18 patients who died with the lesion complex were women. The cause of this difference, if real, is obscure. A similar sex difference is suggested by the report of Spain,[18] who found that in a group of patients with cirrhosis, comprised predominantly of alcoholics, the incidence of jaundice at necropsy was twice as high in females as in males. A predilection for females has also been described in other types of liver disease.¶ It also appeared that this lesion complex may favor young alcoholics. Five of the 18 patients who died with the lesion complex were between 30 and 33 years of age.

Patients with even severe degrees of the hepatic lesion complex recovered, but, when they did, clinical and histological improvement was usually slow. The deep, prolonged jaundice and light or colorless stools sometimes observed made the diagnosis of extrahepatic obstruction appear probable. Indeed, one patient with this lesion complex was explored. In two other patients, laparotomy was obviated by hepatic biopsy, which revealed the characteristic lesion complex. Until actual improvement was evident, some of these patients remained on the brink of hepatic coma for days or weeks, and great care was required not to tip the balance adversely.

The severe acute hepatic disturbance seen clinically in these patients seemed to correlate better histologically with the lesion complex described than with hepatic fat. To be sure, most of the patients with a severe degree of the lesion complex also displayed an abundance of fat in their livers, but several exceptions were extant. Of the 17 patients who died in hepatic coma and were autopsied, all showed a far-advanced lesion complex, but an increase in parenchymal vacuolization was slight in 4 and not evident in 1. It may be noted, however, that these 5 patients were in the hospital for an average of 20 days as compared with 8 days for the other 12 patients. Serial biopsies in three patients with the lesion complex who lived revealed that hepatic fat resolved more quickly than did the lesion complex and even decreased as one patient appeared to become worse. These observations suggest that the five patients with little or no hepatic fat had more on admission but lost some in the hospital, even though they became clinically worse. Previous studies suggest that liver function may get worse while hepatic fat decreases, in the absence of this lesion complex,# and, contrariwise, liver function may improve with no apparent change in hepatic fat content.* In addition, several patients were encountered with large amounts of hepatic fat as essentially the only hepatic lesion who had little functional impairment evident. Thus, although it seems that most chronic alcoholics with cirrhosis drinking up to the time of admission to the hospital have increases in hepatic fat, sometimes marked, it is possible that the fat has little relationship to function.

Unexplained by this hypothesis are the eight patients with icteric indices over 50 who showed no evidence of the lesion complex. Histologically, all had large quantities of hepatic fat except two. The almost complete absence of fat in one of these two patients demonstrates that much functional disturbance may be present with little apparent histological basis. It is possible that the liver cell may exhibit considerable functional impairment before anatomical abnormality is evident and that the livers of these eight patients were in a "pre-lesion" stage. A similar situation

‖ References 18 and 19.
¶ References 17 and 20-22.
# References 2 and 23.
* References 23 and 24.

prevails in the dietary necrotic liver degeneration of the rat, in which the hepatic lesion appears to occur suddenly following a prelesion period of hepatic dysfunction.† But whether these eight patients would have developed the lesion complex is highly speculative, and the possibility remains that their functional disturbance was related to the hepatic fat or to some other factor.

All the patients with the lesion complex had hepatic fibrosis; in one patient it was slight. Whether the fibrosis is a prerequisite for, a result of, or unrelated to the lesion complex cannot be positively stated. Considerable fibrosis has been observed frequently, however, with little or no demonstrable functional impairment, and since a great deal of functional improvement occurred in the patients with serial biopsies without any apparent decrease in fibrosis, the severe acute dysfunction seen in these patients could hardly be attributed to the fibrosis alone.

The cause of the lesion complex remains obscure. Several possibilities, nevertheless, may be considered. Because the disorder represents an acute severe injury to the hepatic parenchyma, the possibility of a viral etiology must be considered, although there is little positive incriminating evidence. Histologically, the lesion complex is quite unlike that seen in viral hepatitis. But it could be argued that a viral infection superimposed on the cirrhosis of the alcoholic may present this histological appearance. It is also possible that alcoholism or malnutrition somehow might activate a latent virus.

Since all the patients with this severe lesion complex were chronic alcoholics thought to be drinking up to the time of admission to the hospital and since this histological picture, to our knowledge, occurs rarely, if ever, in nonalcoholics, the lesion complex may well be the result of the alcohol ingestion, either directly or indirectly. It may be that alcohol directly injures the liver; the blood level of alcohol entering the liver is presumably higher than that going to any other organ. Studies in animals, however, do not favor an hepatotoxic action of alcohol.[26] In man, evidence against an hepatotoxic action includes the demonstration that the administration of alcohol in addition to a nutritious diet to chronic alcoholics with liver disease did not prevent hepatic functional and histological improvement in one patient [27] or exacerbate the liver disease of four patients already treated, even though it was given for as long as 6 to 18 months.[28]

Alcohol may also act indirectly by causing or accentuating a nutritional deficiency, which is a third possible basis for this lesion complex. All the patients with this lesion complex claimed a poor dietary intake prior to admission, and most were undernourished upon entry. In addition, several patients showed associated disorders which are thought to be related to nutritional deficiency, such as Wernicke's syndrome [29] and peripheral neuropathy.[30] No further significant evidence for a nutritional basis for this lesion was derived from the present study. The problem of nutritional liver disease of the rat has recently been partially unraveled by the demonstration of two distinct hepatic lesions, each due to different nutritional deficiencies.‡ The same situation may apply to the human. The apparent independence of the lesion complex described in this study from hepatic fat suggests a different etiological basis for each. In a previous communication,[2] it was also suggested that the cirrhosis of the alcoholic may be related to more than one causative factor on

† Reference 25 and Schwarz, K.: Personal communication to the authors.

‡ References 31 and 32.

the basis of the marked difference in response of the active cirrhosis of a group of alcoholics on similar regimens. In the rat the two separate lesions are fatty cirrhosis and acute necrosis. A very similar fatty cirrhosis is seen in man. Perhaps the lesion complex described in the present study, which in its severe form is an acute necrosis, corresponds to the acute necrosis of the rat, even though there appears to be little histological resemblance. But this comparison at the present time is largely conjectural, as the nature of the liver disease of the alcoholic is far from clear.

Since the cause of this lesion complex is not known, but may be related to a nutritional deficiency, it would seem reasonable to treat patients thought to have it with adequate nutrition. Clinical impressions bear out this idea. Most of such patients have severe anorexia, and the frequent offering of food or even tube feeding may have to be employed. Since many of these victims are wavering on the edge of hepatic coma, any measure which might induce coma should be avoided. Prominent among these deleterious influences are sedatives and hypnotics; certain nitrogenous substances, especially ammonium chloride and large amounts of dietary protein,§ and abdominal paracentesis, which may be obviated by the use of a sodium-restricted diet. Marked disturbances in serum electrolytes should probably be corrected, although such therapy usually has little effect on the clinical course.[35] Vitamins may be administered, although clinical improvement was not observed as a result of this therapy. Parenteral administration of dextrose has been advocated frequently in the treatment of cirrhosis,|| but the present study provided no evidence for any therapeutic benefit in patients with a severe lesion complex. With careful management, many of these patients will probably recover. After recovery, however, the treatment is not concluded and should be directed at what is perhaps both the most difficult and important task of the physician, the permanent withdrawal of alcohol.

SUMMARY

The clinical manifestations and the hepatic histology of 56 chronic alcoholics with liver disease were examined.

Characteristic histological abnormalities consisting of severe degrees of (1) hyaline degeneration (Mallory), (2) liver cell necrosis, and (3) parenchymal disorganization were observed in 18 patients who died with acute hepatic insufficiency. Fibrosis was a constant feature of this pathological picture. Hepatic fat was usually present, but the characteristic lesion appeared to be independent of the amount of fat.

Eleven of these 18 patients were women; all but 1 were jaundiced, often with acholic stools; most had progressive polymorphonuclear leucocytosis, and all died in hepatic coma, usually spontaneous.

Of the remaining 38 patients, most of whom lived, 10 showed the same lesion complex in moderate to marked degree in liver biopsy specimens. Lesser degrees of the lesion complex were usually associated with lesser degrees of acute hepatic functional impairment. Hepatic fat and fibrosis were seen frequently without the characteristic lesion complex and were usually associated with less clinical evidence of active liver disease and a better immediate prognosis.

We conclude that chronic alcoholics may exhibit a typical hepatic lesion complex characterized by hyaline degeneration, liver cell necrosis, and parenchymal disorgan-

§ References 33 and 34.

|| References 36-38.

ization, which is distinct from the fatty lesion and is usually responsible for the clinical evidences of acute severe liver impairment, which may improve slowly but often leads to fatal hepatic coma.

Mr. Vernon E. Taylor, of the Photographic Section, National Institutes of Health, Bethesda, Md., made the photographs.

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POSTPRANDIAL SERUM AMINO ACID LEVELS IN VIRAL HEPATITIS

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LOS ANGELES

IT HAS long been known that the liver plays a major role in the metabolism of amino acids.* It is not surprising, therefore, that abnormally high concentrations of amino acids have been found in the blood of patients with acute yellow atrophy of the liver.[2] The fasting blood amino acid concentration is rarely altered by liver injury less severe than acute or subacute yellow atrophy or some comparable catastrophe.† Individual amino acids, however, may be elevated in less severe hepatic disease.[3] In normal persons, after a high-protein meal the blood amino acid level rises,[4] as does the blood sugar in a glucose-tolerance test, and one might expect that this postprandial amino acid curve would be higher and more prolonged in patients with liver disease. But data bearing upon this hypothesis are scanty. Kinsell and his co-workers have demonstrated an abnormally high blood methionine level in patients with liver disease after the ingestion of methionine.[5] As early as 1908, Jastrowitz reported a similar situation with aminoacetic acid.[6] Kirk found a slightly exaggerated rise in the two-hour postprandial blood amino acid level in patients with liver disease, using a mixture of aminoacetic acid, leucine, asparagine, and tyrosine as a test meal.[7]

In 1924 von Falkenhausen, using a 20 gm. mixture of amino acids as a test meal, found normal or abnormally high postprandial blood levels of amino acid nitrogen in six patients with liver disease "without jaundice," but in six jaundiced patients with liver disease there was little or no rise in the postprandial amino acid nitrogen.[8] This isolated report of von Falkenhausen's seemed inconsistent. Why should the postprandial blood amino acid nitrogen be higher in normal persons than it is in patients with liver disease severe enough to cause jaundice? We were surprised to find, however, in 16 patients with viral hepatitis, that there was indeed a very marked depression of the entire postprandial blood amino acid nitrogen curve.

METHOD

Twenty-two persons without liver disease were used as controls. Half of these were hospitalized and at rest because of peptic ulcer, coronary occlusion, or mild cerebrovascular accident, but all these patients were feeling well and were convalescent at the time of the tests. The other half of the control subjects were healthy ambulatory volunteers. In Table 1 are the liver-

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* Peters and Van Slyck,[1] pp. 793-797.

† Peters and Van Slyck,[1] p. 806.

function values at the time the 16 patients with hepatitis were tested. The diagnosis was made on the basis of the clinical picture and on the laboratory data obtained before and after these amino acid tests were performed.

In each case the test meal consisted of two eggs, a glass of milk, and a piece of toast. This particular meal was used because it is not objectionable to most patients and because it contains a considerable amount (about 20 gm.) of protein. Just before the test meal and at intervals after the test meal of one, two, three, and four hours a blood specimen was drawn.

TABLE 1.—*Liver-Function Tests at Time of Determination of Amino Acid Curves in Sixteen Patients with Viral Hepatitis*

Patient	Age, Yr.	Sex	Serum Bilirubin, Mg./100 Cc.	Cephalin Flocculation	Thymol Turbidity, Units	Alkaline Phosphatase, Bodansky Units
KO	42	M	1.8	+++−	....	10.0
RA	29	M	3.3	++	4.9	7.0
KZ	42	M	0.8	+++	....	6.0
DE	23	M	4.9	++	20.0	...
DR	29	M	1.2	0	4.9	...
TH	25	M	1.7	++++	19.0	...
WR	30	M	1.9	.....	29.0	...
MA	32	M	1.4	.....	8.0	6.0
NO	28	M	5.3	.....	4.0	7.3
NR	41	M	3.0	++++	14.0	6.0
SW	54	M	2.1	++++	20.0	...
BA	62	M	6.5	+++	10.0	...
MN	39	M	14.0	++++	28.0	...
EL	25	F	6.1	++++	18.0	14.5
AL	29	M	1.6	++	13.0	...
SC	34	M	1.7	+++	21.0	...

TABLE 2.—*Statistical Comparison of Differences in Control and Hepatitis Postprandial Amino Acid Curves*

Characteristic of Curve	Control Mean *	Hepatitis Mean	Standard Deviation of Difference Between the Means	t	p
Fasting level	6.3 mg. (22)	6.8 mg. (16)	0.88	0.568	>0.5
Time of maximum rise	1.8 hr. (22)	1.1 hr. (16)	0.30†	2.333	<0.05
Av. % change during 4 hr.	+ 10.0% (18)	− 4.0% (16)	2.19	6.670	<0.001
Highest value, % change	+ 17.6% (22)	+ 2.1% (16)	7.41	2.095	<0.05
Highest value, mg./100 cc.	+ 1.1 mg. (22)	+ 0.5 mg. (16)	0.15	3.637	<0.001
Lowest value, mg./100 cc.	+ 0.1 mg. (18)	− 0.8 mg. (16)	0.21	4.570	<0.001
Lowest value, % change	+ 2.5% (18)	− 11.3% (16)	2.42	5.690	<0.001

* The number of patients is given in parentheses. In four of the control patients one of the blood specimens was lost by accident. These specimens were clearly timed on the falling portions of the curves. Hence these four patients were included only in the computations involving the peak values.

† Computed using Sheppard's adjustment for grouping.

Total amino acid nitrogen levels in the serum of each specimen were determined in duplicate by the copper method of Albanese and Irby.[9] This method measures the copper bound by the alpha-amino nitrogen, two of the histidine nitrogens, the NH_2-N of lysine, and all of the pyrrolidine nitrogen. In naturally occurring mixtures of amino acids the method is accurate within 1 or 2%, and gives very close agreement in duplicate trials.

The urinary excretion of amino acids was measured by the method of Albanese and Irby[10] in six controls and three patients with hepatitis during the 24-hour period encompassing the test and in six controls and six patients with hepatitis also during the period of the test itself.

RESULTS

Among the controls the fasting amino acid nitrogen varied between 5.1 and 8.0 mg. per 100 cc., and among the patients with hepatitis, between 5.6 and 9.7 mg. The means were 6.3 and 6.8 mg., respectively, an insignificant difference (Table 2).

The postprandial curves of the amino acid nitrogen, however, were strikingly different, as shown in Chart 1. When the individual curves are plotted on one graph, it is difficult to judge the significance of the variation and overlapping. Hence, in Table 2 is recorded a statistical comparison of various characteristics of the curves. As one might guess from Chart 1, postprandial serum amino acid nitrogen concentration among the patients with hepatitis differed significantly from that of the controls in the following respects: (*a*) The highest postprandial value was attained earlier. (*b*) The highest value was lower, expressed either as milligrams per 100 cc. or as percentage difference from the fasting level. (*c*) The maximum fall was

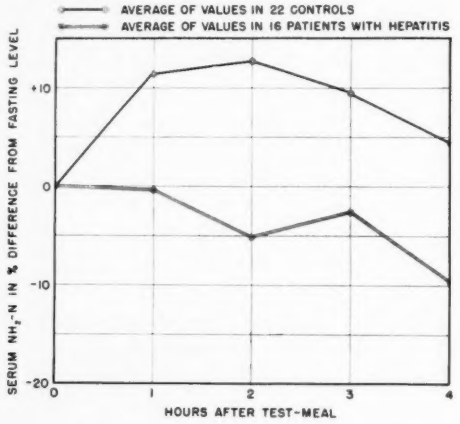

Chart 1.—Serum amino acid nitrogen after ingestion of 20 gm. protein meal.

TABLE 3.—*Characteristics of Amino Acid Curves in Five Patients with Hepatitis During and After Illness*

Patient	Serum Bilirubin at Time of 1st Test, Mg./100 Cc.	Av. 4 Hr. Amino Acid Level, % Change		Difference Between Tests, % Change
		1st Test	2nd Test	
MA	14.0	−6.66	+ 6.50	+13.16
TH	1.7	−3.50	+15.25	+18.75
NO	5.3	−7.00	− 2.50	+ 4.50
SC	1.7	−0.33	+13.50	+13.83
RA	1.2	−8.75	− 0.75	+ 8.00

Mean difference between tests = + 11.65

Standard error of difference = 2.47; $t = 4.71$; $p = <0.025$

greater, expressed either as milligrams per 100 cc. or as percentage difference from the fasting level. (*d*) The average percentage difference from the fasting level during the four-hour period was lower.

The characteristic showing the most significant difference between controls and patients with hepatitis was the average percentage change from the fasting level throughout the four-hour period. The fiducial limits at the 5% probability level were therefore determined for this characteristic and were found to be $+ 10 \pm 14\%$ of the fasting level.

Five of the 16 patients with hepatitis were retested after the serum bilirubin, cephalin flocculation and/or thymol turbidity, and serum alkaline phosphatase values had become normal and the patients were clinically well. In each case the average four-hour level was within the fiducial limits, as shown in Table 3. These limits were

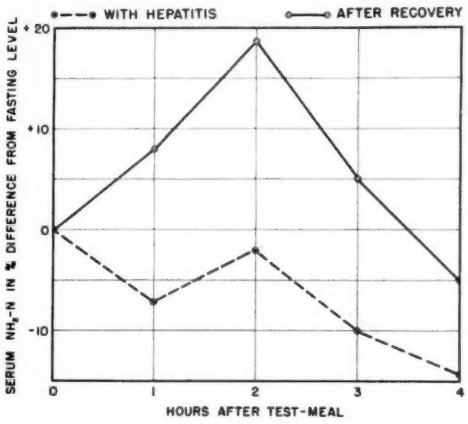

Chart 2.—Serum amino acid nitrogen after ingestion of 20 gm. protein meal in one patient.

TABLE 4.—*Miscellaneous Patients*

Patient	Diagnosis	Serum Bilirubin, Mg./100 Cc.	Average 4 Hr. Level, % Change *
PO	Cirrhosis	2.5	+ 4.50
WA	Cirrhosis	10.7	+ 16.50
CA	Cirrhosis	38.6	+ 8.50
CO	Cancer of pancreas	10.0	+ 10.50
WI	Cirrhosis	1.2	+ 2.50
BE	Ulcerative colitis; nephrosis; hypoalbuminemia	0.8	— 1.25
BZ	Cirrhosis	1.0	+ 7.00
TE	Cirrhosis	0.8	— 8.00

* Fiducial limit ($p = 0.05$) is $+ 10 \pm 14\%$.

TABLE 5.—*Urinary Excretion of Amino Acids During Postprandial Period*

	Mean Amino Acid Nitrogen, Mg.		Standard Deviation of Difference Between Means	t	p
	Controls Group	Group with Hepatitis			
4 hr.	50 (6 patients)	54 (6 patients)	18	0.2156	>0.5
24 hr.	255 (6 patients)	323 (3 patients)	179	0.3778	>0.5

exceeded in only three of the five curves when the hepatitis was active. Two of the patients had low normal curves. The levels in all five, however, changed in the direction of normal, and the degree of average change was such that the likelihood of its occurrence by chance would be less than 1 in 40 (t=4.71). Chart 2 illustrates these changes in one of the patients (MN). A few patients with other diseases that might

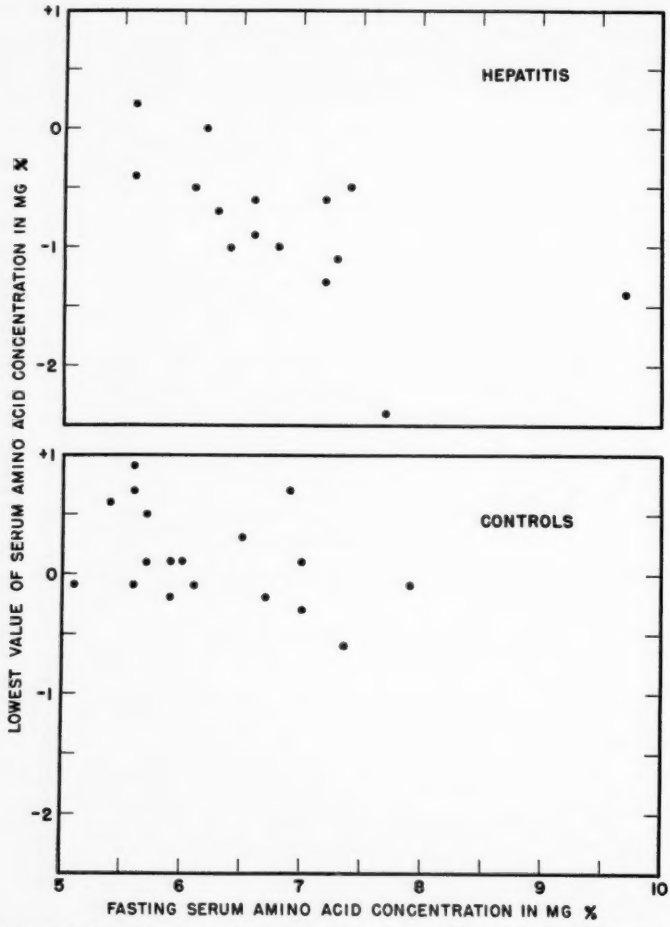

Chart 3.—Relation of fasting amino acid levels to the maximum fall, in milligrams per 100 cc.

theoretically have influenced the test were excluded from the control series, although all but one of them revealed values within normal limits, as shown in Table 4. One patient with Laennec's cirrhosis had a significantly low curve, like those of the patients with hepatitis.

The average urinary amino acid nitrogen among the controls was 50 mg. for the 4 hours of the test and 255 mg. per 24 hours. Comparable values among the patients with hepatitis were 54 and 323 mg., respectively. As shown in Table 5, these were not significant differences.

It seems strange (Table 2) that the difference between the peak levels was more significant when expressed as milligrams per 100 cc. than when expressed as percentage of fasting level, whereas the converse was true of the maximum fall. This suggested that the factors responsible for the fall in the amino acid level were more closely related to the fasting levels in the patients with hepatitis than in the controls, or vice versa. This hypothesis was clearly substantiated by the scatter graphs relating the fasting levels to the maximum falls (Chart 3). The correlation coefficient (r) was significant for the relation between the fasting level and the maximum fall in the patients with hepatitis but was not significant at all for the relation between the fasting level and the maximum rise in either group or for the fasting level and the maximum fall in the control group (Table 6). There remained the possiblity that a relationship between the factors tending to depress the normal curves and the normal fasting values was obscured by the factors tending to make the normal curves rise.

TABLE 6.—*Correlation Coefficients*

	r	t	p
Controls: fasting level and absolute peak amino acid concentration.....	0.2127	0.8909	Approx. 0.4
Controls: fasting level and absolute maximum fall.....	0.4103	1.8000	Approx. 0.1
Patients with hepatitis: fasting level and absolute peak.....	0.3101	1.2301	Approx. 0.25
Patients with hepatitis: fasting level and absolute maximum fall.....	0.7201	3.9011	<0.005
Controls: peak amino acid concentration and maximum fall.....	0.4255	2.0781	Approx. 0.05
Patients with hepatitis: peak amino acid concentration and maximum fall..	0.5898	3.6181	<0.005

TABLE 7.—*Regression Coefficients* (b) *(Influence of Peak Amino Acid Concentration upon Maximum Fall in Amino Acid Concentration) and* t *Value for Difference Between* b *for Controls and Patients with Hepatitis*

	b	t	p
Controls	0.2084	1.090	>0.25
Patients with hepatitis.....	0.2918		

Hence r was determined for the relationship between the peak value in each curve and the maximum fall. Among both the patients with hepatitis and the controls this figure was significant (Table 6), although it was more significant for the patients with hepatitis. The regression coefficients (Table 7) reveal an insignificantly greater effect upon the hepatitis curve than upon the normal curves by the factor related to the peak levels.

COMMENT

The data reported and statistically analyzed justify the following conclusions: 1. After the 20 gm. test-meal used, the serum amino acid curve in hepatitis is depressed as compared with the normal. 2. This depression of the postprandial amino acid curve is not due to excessive urinary loss. 3. The extent of the fall of both the control and the hepatitis curves is positively correlated with the peak amino acid level, while no such correlation exists for the rise in either kind of curve. 4. The factor that tends to depress the postprandial amino acid curve is, in the patients with hepatitis, either more powerful than it is in the normal patients or less vigorously opposed.

What could this factor be? It is impossible to tell from the data collected thus far, but it may be of interest to consider a hypothesis. Insulin, which must be secreted after the breakfasts served, is capable of lowering the serum amino acid concentration as promptly and definitely as it does the blood sugar.[11] Neither the shape of the normal curves nor the relation of the fall to the height of the curve would be inconsistent with the action of insulin. There is an apparent discrepancy between the findings reported here and the results of other investigators, who found that certain amino acids reached a higher serum level in patients with liver disease after the ingestion of these amino acids. This difference might conceivably be due to differences in the amount of insulin secreted in response to a fairly normal breakfast and in response to the ingestion of purified amino acids.

If insulin were the factor causing the curves to fall, the excessive lowering of the curves in liver disease might be due to an increased susceptibility to insulin, as, for example, if the patients with hepatitis were unable to release amino acids into the blood stream at a normal rate. In considering this possibility, it was at first suspected that differences in the curves were due to excessive ingestion of protein, a hazard to which a hospitalized patient with hepatitis is usually exposed. Could exorbitant protein consumption have an effect upon the amino acid curve analogous to the effect upon the glucose-tolerance curve of excessive eating of carbohydrate?[12] It soon became evident, however, that the depressed curves were found in patients recently nauseated and underfed as well as in those whose appetites were ravenous.

An increased susceptibility to insulin might also arise from poor absorption of amino acids or from poor digestion of protein itself. While amino acid infusions are necessary to test this alternative, it seems unlikely that liver disease alone would alter protein digestion or absorption except through abnormalities in gastrointestinal motility. Yet most of the patients with hepatitis tested had a good or excellent appetite, and none had diarrhea.

There is another way in which insulin might be responsible for the depressed curves in hepatitis. Suppose the liver were capable of destroying insulin. There might then be a lesser destruction of insulin by the diseased liver and thus a higher insulin blood level. It has long been known that insulin is destroyed in the body,‡ and it has been demonstrated that homogenized liver tissue destroys insulin in vitro.§ If insulin is destroyed by the liver, and is destroyed less readily by a diseased liver, one might perhaps more easily explain certain aspects of hepatic hypoglycemia. The latter is sometimes paroxysmal, as in an islet cell tumor, and in some cases it even occurs at the precise period of the glucose-tolerance curve that one anticipates the peak action of insulin.[14]

Finally, it is theoretically possible that an excess of insulin is secreted in patients with hepatitis. Failure to store glycogen properly, for example, might allow the postprandial blood sugar to rise more sharply, and the relative hyperglycemia would then initiate a greater secretion of insulin.

SUMMARY

Changes in the serum amino acid concentration were followed after a standard 20 gm. protein meal in 22 controls and in 16 patients with viral hepatitis.

‡ Peters and Van Slyck,[1] pp. 230-231.

§ Reference 13 and Jenden, D., and Taylor, D.: Unpublished data.

As compared with the control curves, the amino acid curves in the patients with hepatitis reached a lesser peak more quickly and fell more rapidly to a lower level.

Five of the patients were retested after recovery from hepatitis. These curves were within normal limits.

The urinary excretion of amino acids was no higher in the patients with hepatitis than in the controls.

In a few other patients with altered liver function, including six with Laennec's cirrhosis, the amino acid curves were normal, but in one patient with cirrhosis a low curve was found.

The activity of the factor causing the curves to fall is positively correlated with the peak concentration of amino acids, and this factor is either more active or less effectively opposed in the patients with hepatitis than it is among the controls.

It is suggested that this factor may be insulin.

Drs. Robert Buckley, John Evans, Marvin Gaster, Earl Dore, Stanley Marcus, and other resident physicians assisted in this study.

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Treatment in Internal Medicine

TREATMENT OF AMEBIASIS

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THE HISTORY of the chemotherapy of amebiasis has been one of high hopes and high "cure" rates. One new drug follows another. Each triumph has been tempered by sobering experimental evidence that the ideal amebacidal drug has not been found. The clinician confronted with the very practical problem of selecting the most effective treatment for an individual patient with amebiasis is unlikely to find secure support in the conflicting reports of the recent publications for his therapeutic regimen. Even though there is an intensive search for more effective agents, a high cure rate can be expected with the proper use of the drugs we have. I propose to discuss the basis for therapy in the light of current understanding of the pathogenesis of amebiasis, the pharmacology of antiamebic drugs, and the clinical experience of many investigators. Extensive bibliographies dealing with the background of treatment of human amebiasis are in current reviews and papers.*

The clinician treating amebiasis should know the factors which make evaluation of chemotherapeutic agents difficult. He should be aware of the therapeutic failures that occur under the most careful management. Amebiasis is a chronic disease with variable host resistance. Also the pathogenicity of strains of amebae varies. Little is known of the mechanism responsible for invasion of the human host. Ordinarily man puts up little visible tissue defense against Endamoeba histolytica. The bacterial flora of the intestine, the state of nutrition, and repeated infection play at least an auxiliary role in the invasion of the host. There is evidence that spontaneous "cure" occurs without treatment.[6]

Throughout this discussion the word "cure" is predicated on the fact that careful clinical and laboratory studies have been negative over a period of many months. It is understood that an asymptomatic infection may still exist under these circumstances.[4] Acute and recently acquired infections are more easily cured than those of great chronicity. The success of an antiamebic drug in the laboratory is not necessarily indicative of high efficacy in clinical trials. Likewise, a drug may be very effective in clinical experiments after it has made a poor showing in the laboratory. The actual site and manner of action of drugs has not been established. For example, certain antibiotics are thought to alter the bacterial flora upon which the amebae are dependent for survival; others act directly on the parasite. Furthermore, most antiameba drugs are far more effective on the bowel phase of infection though a few destroy the organisms chiefly deep in the tissues.

The testing of drugs has been very difficult to evaluate. There has been no way to establish accurately the proper dose and duration of therapy of drugs on a comparative basis. Furthermore, the testing of drugs in patients has been hindered by inherent

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* References 1-5.

difficulties in setting up controlled experiments. The problems of careful follow-up studies, spontaneous remission or cure, strain difference, host susceptibility, and external environment make it difficult to compare the reported results of therapy. Thus, with the introduction of each new agent the clinician must maintain a highly critical attitude. Because of strain differences he must wait patiently for the results of thorough and extensive clinical trials in different parts of the world.

The general principles of therapy are based on the pathogenesis of the disease, the known pharmacological action of the drugs, and clinical experience. We know that an infection with E. histolytica is characterized by primary localization in surface ulcers and in the wall of the large bowel. Secondary or metastatic invasion of deep tissues of the bowel, its capillaries and lymphatics, the liver, and other organs occurs. Thus, one may divide the infection into the superficial bowel phase and the deep tissue phase. The metastatic hepatic amebiasis may be the only clinically recognized active form of disease. The colonic phase may be active; or it may exist with an unrecognized amebic hepatitis.

From the standpoint of treating the individual patient the clinician must make the tacit assumption that the bowel and tissue phases coexist. He must also recognize that an infection of the host exists if cysts of E. histolytica are being passed in the stools. The cysts are not casual interlopers living in the gut.

The drugs available for treatment fall well into two major classes—those effective in clearing the bowel infection and those killing the parasite deep in the tissues. The two effects are explained partially by differences of concentration of the drugs in bowel and deep tissue.

To insure as high a cure rate as possible, both kinds of drugs should be used at the same time in therapy—one directed at tissue infection and another at parasites in the bowel. This is referred to as combined therapy (dual, concurrent, or mixed therapy). For the past 35 years English physicians have used a preparation for oral administration containing emetine, bismuth, and iodide (E. B. I.), and very early "cure" rates of 90% were reported. This combination of drugs has stood the test of time and is still standard therapy. Manson-Bahr and Adams and Maegraith † mention the advisability of combining other drugs with emetine, bismuth, and iodide therapy in treatment of chronic cases. In recent years American investigators have concentrated their work on the effectiveness of single agents in the therapy of amebiasis. By and large, the relapse rates with single drugs have been disappointingly high. Combinations of emetine, chloroquine, or quinacrine (Atabrine) with diiodohydroxyquinoline (Diodoquin), iodochlorhydroxyquin (Vioform), carbarsone, or oxytetracycline (Terramycin) appear to be more effective than any single agent.‡ The attack on the problem of treatment failure has been furthered by repeating courses of therapy at close intervals and changing the drug used with each course.

The recommended management insuring the highest "cure" rate in treating an individual person sick with clinically recognized amebiasis includes repeated courses of some form of combined therapy. The precedent for combined therapy has been established. There has been a trend, accentuated by the wishful thinkers who abstract medical literature or with drug house brochures, to emphasize "the new drug for treatment of amebiasis." The studies reported usually represent experimental pro-

† References 7 and 8.

‡ References 5 and 7-13.

grams, and adequate attention is not called to treatment failures. The fact that another chemical agent is found tc have antiamebic qualities is not tantamount to accepting or recommending it for standard therapy. I wish to make clear that there is the pragmatic problem of treating the patient with a tried therapeutic regimen and the independent experimental problem of treating larger groups. In this review I have been primarily interested in the former problem.

In choosing drugs for therapy it is fair to state that one drug is not necessarily superior to another. The choice will be based on a number of factors, such as the relative toxicity, the specific response of patient, the cost of drug, the desirability of ambulatory treatment, and the physician's experience with a compound.

As part of the management of the patient the physician has the responsibility of investigating the possible sources of infection, not only as a public health measure but to protect against reinfection and "therapy failure."

TREATMENT OF TISSUE AND BOWEL PHASES

Treatment of Tissue Phase.—The available drugs primarily effective against the tissue phase of amebiasis are chloroquine, emetine, and quinacrine. Chloroquine was recognized as an extremely effective antiamebic drug in 1948 by Conan,[14] and it has largely replaced parenterally administered emetine. This discovery is the most significant advance made in the practical chemotherapy of amebiasis in recent years. Chloroquine has proved to be highly effective in therapy of amebic abscess of the liver, hepatitis, pleural amebiasis, draining sinuses, and abscess of the peritoneum. Chloroquine is now considered the drug of choice for the treatment of extraintestinal amebiasis. It has a few side-effects, consisting of occasional headache, blurred vision, gastrointestinal upsets, and irritability of the central nervous system. Most patients tolerate it well. Therapy can be given to the ambulatory patient. The dose is 1.0 gm. of chloroquine (Aralen) diphosphate daily for 2 days, and then 0.5 gm. is given for 19 days. If toxic symptoms appear, use of the drug is discontinued for 24 to 48 hours and then is resumed. This drug has little amebacidal activity in the intestinal tract.

Emetine is a powerful cytotoxin. It may injure skeletal or heart muscle. Profound weakness and hypotension may be produced by its use. It may cause diarrhea, nausea, and vomiting. A patient under treatment with emetine should be in bed. The parenteral use is limited to the severely ill patient with amebic abscess or fulminating colonic infection. The dose of emetine hydrochloride in an adult should not exceed 65 mg. injected subcutaneously each day for not more than seven days. Serial EKG tracings are recommended. If significant T-wave changes occur, the drug should be stopped. Electrocardiographic changes have been observed up to 14 days after emetine has been discontinued. Emetine is not effective in curing intestinal amebiasis.

Quinacrine has been used successfully in treatment of extraintestinal amebiasis by Radke.[13] When it was used concurrently with carbarsone cure rates were found to be as high as with other forms of combined therapy. Quinacrine was recommended in doses of 0.1 gm. four times a day for 15 days.

Treatment of Bowel Phase.—The iodoquinoline compounds diiodohydroxyquinoline, iodochlorhydroxyquin, and chiniofon; the arsenicals carbarsone, glycobiarsol (bismuth glycolylarsanilate), and thiocarbarsone, and the antibiotics oxytetracycline

and fumagillin are all effective agents in treatment of amebic infection of the colon. As stated before and emphasized again at this point, it is impossible with existing data to single out any agent as being "the best." With each, failures are to be expected.

The iodoquinoline compounds have a very low incidence of toxic reactions, although nausea, abdominal cramps, vomiting, anorexia, diarrhea, and iodism may be encountered. Diiodohydroxyquinoline is least likely to have side-effects. The initial course of therapy with these drugs is recommended as follows: diiodohydroxyquinoline is given as a 650 mg. tablet three times a day for 15 to 21 days. Chiniofon is recommended in 0.25 gm. doses four times a day for a minimum of 10 days. Chiniofon is suitable for administration in retention enemas. Four grams of chiniofon is dissolved in 200 ml. of water and slowly introduced into the rectum. The solution is retained up to eight hours. Iodochlorhydroxyquin is given 0.25 gm. doses (in gelatin capsules) four times a day for a minimum of 10 days.

Carbarsone, glycobiarsol (Milibis), and thiocarbarsone are the drugs of choice of several arsenicals. These compounds have severer toxic reactions, such as encephalitis, neuritis, vomiting, diarrhea, dermatitis, and injury to an already damaged liver or kidneys. The risk is slight if the drugs are stopped at first sign of toxicity. It has been demonstrated that the liver-function studies improved or that there was no detectable damage to the liver during arsenical therapy.§ Carbarsone has been used most widely. To date glycobiarsol and the thioarsenites have not proved to be any more effective than carbarsone. The oral dose of carbarsone is 0.25 gm. three times a day for 7 to 10 days. As a retention enema, 2 gm. is dissolved in 200 ml. of 2% sodium bicarbonate solution and given rectally on alternate nights for five injections. Glycobiarsol is given in 0.5 gm. doses three times a day for 7 to 10 days. Thiocarbarsone has been used in 100 mg. doses three times a day for 10 days.

The antibiotics have been widely praised as very effective antiamebic agents. With more experience they have not proved to be ideal amebacides. One need only recall the rapid rise of chlortetracycline (Aureomycin) to prominence and the subsequent fall into discard. When one is not concerned with an experimental program but is treating individual patients for amebiasis, antibiotics should not be used alone. More extensive trials are needed. Martin, Garfinkel, Brooke, Weinstein, and Frye [6] and McHardy and Frye [5] have written the most recent reviews of the use of antibiotics in the treatment of amebiasis. These articles should be consulted for a complete perspective.

Chlortetracycline, oxytetracycline, bacitracin, neomycin, erythromycin, and fumagillin all have antiamebic qualities, either by direct action on the amebae or by action on the bacterial flora sustaining growth of the protozoan. Oxytetracycline and fumagillin are the most promising antibiotics and have good records with low relapse rates in the control of colonic amebiasis. They are particularly effective when acute bowel symptoms or dysentery are present. Oxytetracycline has been given in doses of 500 mg. every six hours (2 gm. a day) for 10 days. A fairly high incidence of drug-induced diarrhea and severe stomatitis has been observed, and so it is not good for ambulatory treatment. Fumagillin, which has a direct action on the amebae, is given in doses of 20 mg. three times a day for 10 days. It has few and minor toxic effects. Further experience is required to determine its place in the therapy of amebiasis.

§ References 3 and 15.

The combination of emetine, bismuth, and iodide in enteric-coated tablets represented the first real attempt at combined therapy and is highly effective. Other single courses of therapy have not proved to be superior to emetine, bismuth, and iodide, a point often overlooked by American writers. The drawbacks to the use of emetine, bismuth, and iodide are the toxic reactions of nausea, vomiting, irritation of colon, and weakness. It can not be used for ambulatory patients. The dose is a 65 mg. tablet given morning and evening for 10 days.

GENERAL MANAGEMENT

A plan for management of the patient sick with amebiasis must be individualized. There is no one best course of therapy. The guiding principles are those of combined therapy and repeated courses. Patients can be divided into three major groups:

1. The group with mild to moderate disease of the colon and liver can be treated on an ambulatory basis with the safer and more inexpensive drugs. The first course may consist of chloroquine combined with an arsenical or an iodoquinoline compound. During the next several weeks I use two or more additional courses, possibly changing the agent for control of the bowel phase of disease. Recurrences and residual amebic ulcers of bowel can be treated with antibiotics or retention enemas of chiniofon or carbarsone.

2. The sicker patients, with diarrhea, hepatitis, and systemic manifestations of the disease, may need treatment in bed or hospital. Otherwise they can be managed as previously outlined. If the dysentery is severe or the patient is acutely ill, antibiotics may be used in addition. In some instances emetine may be required to stop the severe illness.

In certain instances of chronic amebiasis, in severe dysentery, and during antiamebal treatment when amebic ulcers become bacterially infected, secondary bacterial invaders may contribute to symptoms. Sulfa drugs, penicillin,[6] chloramphenicol, and other appropriate antibiotics are useful. They may be life saving in combating the effects of nonamebic infection.

3. Patients with amebic abscess constitute another group. First treat by drugs. Some abscesses will heal with drug therapy. The drug of choice is chloroquine, but emetine may be used initially for the sickest patients. Abscesses which do not respond to chemical treatment must be treated by needle aspiration or surgical drainage. In any event, antiamebic drugs and antibiotics should be administered before surgery is attempted. Drainage must be done under sterile conditions, for mortality rises considerably following bacterial contamination. Reference should be made to the papers by DeBakey and Ochsner [17] and Sodeman, Doerner, Gordon, and Gillikin [11] for the management of amebic abscess.

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Progress in Internal Medicine

RECENT ADVANCES IN NUTRITION AND METABOLISM

II. Review of the Literature on Vitamins, 1952

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A SMALL monograph entitled "Present Knowledge in Nutrition" has been published recently.[1] This should prove a most useful reference; sections dealing with calories, protein, fat, carbohydrate, minerals, and vitamins are included.

VITAMIN A

Cama and associates * reported further studies of the characteristics of vitamin A and related compounds. Kaiser and Kagan [6] have described a technique for the determination of vitamin A in feces.

Prolonged administration of quinacrine (Atabrine) to rats was found to result in damage to the liver and impairment of the ability of this organ to store vitamin A. Absorption of vitamin A and carotene was depressed by the drug.[7]

The administration of excessive amounts of vitamin A to rats and other animals accelerates the growth and maturation of epiphyseal cartilage cells and the remodeling process which accompanies bone growth. Such effects are suggestive of hormonal action, possibly that of the anterior pituitary. However, studies by Wolbach and Maddock [8] in hypophysectomized rats have shown that these effects occur in the absence of the pituitary and in spite of marked atrophy of the adrenal glands. Similar effects have been observed upon the addition of excessive amounts of vitamin A to the medium containing tissue culture of bone from chick embryos.[9] The changes observed in tissue culture are qualitatively similar to those seen in animals made hypervitaminotic by feeding large amounts of vitamin A.† The use of tissue culture techniques appears to offer distinct advantages in the study of the effects of vitamin A upon tissues.[14]

An unusual case of vitamin A deficiency in a 5½-month-old infant was reported by Cornfeld and Cooke.[15] Facial palsy and increased intracranial pressure observed in this patient might possibly be attributed to compression of tissues of the growing central nervous system by failure of normal growth of the axial skeleton, which has been observed in various mammalian species and, more recently, in the chicken and duck.‡

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* References 2-5.

† References 10-13.

‡ References 10, 11, and 13.

Kirk and Chieffi [16] observed the effects of vitamin A administration on plasma vitamin A concentration, conjunctival changes, dark adaptation, and follicular hyperkeratoses in a group of 23 subjects with low plasma vitamin A levels. Mean plasma vitamin A values rose during administration of 30,000 units of vitamin A acetate daily, dropped sharply when therapy was discontinued, and returned to approximately pretreatment values within eight weeks. Definite beneficial effects of this therapy upon localized conjunctival thickening, blepharoconjunctivitis, and "toad skin" were observed. Follicular hyperkeratosis of the forearms persisted in five of eight patients, and improvement in dark adaptation was observed in only 4 of 17 subjects.

Kaser and associates [17] reported that patients with a sprue-like syndrome showed no increase in serum vitamin A levels after oral administration of the vitamin given in either the ester or the alcohol form. Patients with chronic pancreatic disease showed no change in serum levels of the vitamin after administration of its ester form, but they were found to have an increase in serum vitamin A after administration of the alcohol form.

Steigmann and co-workers [18] confirmed the previously reported interference with absorption of vitamin A by mineral oil given close to mealtimes. However, the usual therapeutic dose (30 cc.) of the oil given at bedtime was without effect upon plasma vitamin A levels.

Kagan and Kaiser [19] found high concentrations of vitamin A in the livers of three patients with the nephrotic syndrome. This finding, in conjunction with the previously reported observation of elevated plasma vitamin A levels in this syndrome, suggests impaired metabolism of vitamin A in nephrosis.[20]

Stoesser and Nelson [21] have reported that synthetic vitamin A palmitate can be safely given to children allergic to or nontolerant of fish liver oils and is of value in treating the dry and ichthyotic skin which may underlie infantile eczema. A comparative study of the toxicity of aqueous and oily preparations of vitamin A has been made by Lewis and Cohlan.[22] Additional cases of hypervitaminosis A have been reported.§

THIAMINE

Burch and her associates [26] have reported a microfluorometric method for the measurement of thiamine and thiamine phosphate in small quantities of blood and blood cells. Teeri [27] has proposed a fluorometric method for the determination of thiamine, employing cyanogen bromide. Bessey and co-workers [28] reported a modification of the thiochrome method for the determination of thiamine in urine.

Studies of blood cocarboxylase levels in normal and diabetic subjects before and after the administration of insulin strongly suggest that insulin is necessary for the normal phosphorylation of thiamine.[29]

Phillips and associates [30] studied the effect of administration of glucose and saline solution, thiamine, and other vitamins in the treatment of Wernicke's syndrome. They concluded that the ophthalmoplegia of this syndrome is related to a specific lack of thiamine; nystagmus and ataxia also appeared to be related to thiamine deficiency, but the evidence for this was less conclusive. No conclusion could be reached regarding the relationship of mental symptoms to deprivation of thiamine or other vitamins. In two cases a definite increase in ocular paralysis

§ References 23-25.

occurred after the administration of a mixture of multiple vitamins exclusive of thiamine, suggesting the possibility of an accentuation of thiamine depletion by such administration. Griffith [31] found that patients with beriberi who survive the acute stages of the disease and whose condition is properly managed do not have evidence of residual clinical heart disease and that the incidence of cardiovascular disease of other causes is no higher among such recovered patients than among the general population.

The literature pertaining to thiamine intolerance was reviewed by Jaros and associates,[32] who also reported two cases observed by themselves. On the basis of these data and animal experiments they concluded that thiamine overdosage produces a simple chemical toxicity, principally through the formation of excessive amounts of acetylcholine and histamine. Epinephrine (Adrenalin) was found to be an effective antidote to such toxicity.

RIBOFLAVIN

Suvarnakich and co-workers [33] investigated the riboflavin content of the serum of 141 normal subjects and found mean values for free serum riboflavin and flavin-adenine dinucleotide of 0.84 and 2.32 γ per 100 cc., respectively. The error of the microfluorometric technique employed was found to be relatively slight, and the authors suggested that serum riboflavin values, especially for flavin-adenine dinucleotide, might be of value in the diagnosis of human riboflavin deficiency. Maynard [34] reported that in 25% of a group of 148 patients with psoriasis who were treated with daily oral supplements and weekly injections of riboflavin the psoriasis healed completely and that in a large number there was definite improvement.

NICOTINIC ACID

Nicotinic acid functions in the body as coenzymes I and II, which are diphosphopyridine nucleotide and triphosphopyridine nucleotide, respectively. Many studies of coenzymes and pyridine nucleotides in blood and tissues have been reported. Recently, a method for differential assay of pyridine nucleotides and N^1-methylnicotinamide in small samples of blood has been developed [35] and the pyridine nucleotide partition in erythrocytes has been investigated.[36]

The finding that tryptophan is converted to nicotinic acid compounds in man, as in many other species, has been discussed in previous reviews.|| Recent investigation of the pathway of conversion in rats, using tryptophan doubly labeled with deuterium and N^{15}, supports previous postulations that tryptophan is converted to kynurenine, 3-hydroxyanthranilic acid, and quinolinic acid.[39] Quinolinic acid, in turn, can be converted to nicotinic acid. Mason and Berg [40] found that in liver slices anthranilic acid was formed from *l*-tryptophan and *l*-kynurenine. They noted impaired conversion of tryptophan in tissue homogenates from pyridoxine-deficient animals, in confirmation of tests by others. The role of the kidney in the metabolism of tryptophan was discussed. Reddi and Kodicek [41] found pyruvic acid, kynurenine, and 3-hydroxyanthranilic acid in human urine after tryptophan administration. Their findings suggest that the conversion of tryptophan to nicotinic acid follows the same pathway in man as in the rat.

|| References 37 and 38.

Since tryptophan serves as a precursor of nicotinic acid, the requirement of this vitamin can be determined only in relation to tryptophan intake. Goldsmith and associates [42] reported that pellagra developed in each of three subjects receiving a "corn" diet which furnished approximately 5 mg. nicotinic acid and 200 mg. tryptophan daily. In one subject who received the same diet supplemented with 2 mg. of nicotinamide no deficiency was observed. Likewise, deficiency was not induced when a wheat diet which furnished about 6 mg. of nicotinic acid and 230 mg. of tryptophan was administered. In subsequent studies, wheat diets containing essentially the same amount of nicotinic acid and tryptophan as the corn diets were given to three subjects.[43] Nicotinic acid deficiency developed in only one instance, and in all subjects excretion of nicotinic acid metabolites decreased more slowly when wheat, rather than corn, was the staple cereal of the diet. From these data, the minimal daily nicotinic acid requirement was considered to fall between 5 and 7 mg. when the diet furnished 200 mg. of tryptophan. The requirement appeared to be greater on the corn than on the wheat regimen. Supplementation of the corn diet with increments of nicotinamide showed that when the diet furnished more than 8 to 10 mg. of nicotinic acid daily, proportionately larger amounts of nicotinic acid metabolites were excreted in the urine than at lower intakes, suggesting an adequacy of nicotinic acid stores at this level.

Clinical findings in experimentally induced nicotinic acid deficiency included pellagrous dermatitis, glossitis, diarrhea, and mental depression.[42] In addition, cheilosis, angular stomatitis, and nasolabial seborrhea were observed, signs previously noted in riboflavin deficiency and following administration of a pyridoxine antagonist. That similar findings can occur in deficiency of several B-vitamins attests the close relationship of these factors in metabolic processes.

The role of corn in the pathogenesis of pellagra may not be explicable solely by its low tryptophan content. Cravioto and associates [44] have confirmed earlier studies which showed that lime-treated corn will prevent tryptophan-nicotinic acid deficiency in rats when substituted for raw corn in diets low in these nutrients. Laguna and Carpenter [45] attributed the effects of lime treatment to release of nicotinic acid precursors which were otherwise unavailable. Cravioto and associates [44] suggest that certain amino acids in corn may be destroyed or racemized by alkali treatment, thus correcting some amino acid imbalance. Numerous studies in rats on low-casein diets have shown that amino acid imbalance can lead to nicotinic acid deficiency. In explanation of this, it has been suggested that with low-casein diets certain amino acids are more limiting for protein synthesis than is tryptophan.[46] When these amino acids are added, tryptophan becomes limiting and is drawn into protein synthesis at the expense of nicotinic acid formation.

Lojkin and associates [47] reported a significant increase in the excretion of N^1-methylnicotinamide (N^1-Me) with advance in pregnancy. There was a corresponding increase in excretion of the 6-pyridone of N^1-Me in the instances in which this metabolite was measured. The authors stated that total excretion of nicotinic acid compounds averaged more than the sum of nicotinic acid consumed and that which could be formed from dietary tryptophan. Complete data in support of this statement were not given, and the method for computing the amount of nicotinic acid which might be furnished from tryptophan was not included.

The increase in excretion of N^1-Me during pregnancy could not be entirely accounted for by the added metabolism of the fetus and placenta, as it often per-

sisted after delivery. It seems possible that hormonal influences were important. Hormones have been shown to influence N^1-Me excretion in dogs.[48] Beher and associates [49] obtained prolonged reduction of N^1-Me excretion in female rats receiving 0.5 mg. testosterone propionate daily, intramuscularly. Increase in hormone dosage to 6.25 mg. daily resulted in a fall in N^1-Me excretion followed by a rise to about twice the control value. Testosterone increased the concentration of coenzymes I and II, as well as organ weight, in the liver but not in the kidney. The assumption that diminished conversion to N^1-Me provided nicotinic acid for increased synthesis of coenzymes was tenable in some experiments but not in others. It can be anticipated that further studies of hormone-vitamin relationships will elucidate this and many other metabolic problems.

Nicotinic acid compounds continue to receive attention as therapeutic agents in diverse conditions. Nicotinic acid has been reported to be beneficial in psychoses of senility when given in large doses.[50] Benefits would appear to be due to pharmacologic rather than vitamin action. Kaufman [51] noted improvement in joint mobility when 1,500 mg. of nicotinamide was administered daily, with regression after therapy was discontinued. Nicotinic acid was given to patients with dysmenorrhea in doses sufficient to produce flushing, on the basis of the assumption that vasospasm was the mechanism producing pain.[52] Of 40 patients followed for more than six months, 35 obtained "satisfactory" relief. A high-protein diet, including 1 qt. (0.95 liter) of milk daily, was also prescribed.

Intravenous injection of 100 mg. of nicotinic acid produced marked leucopenia, chiefly affecting the granulocytic series, in normal and splenectomized subjects.[53] This effect was considered to be related to vasodilator action.

An ointment containing nicotinamide was reported to have antipruritic properties in dermatoses of varied etiology and to restore the normal condition of the skin in patients with hyperkeratosis.[54]

PYRIDOXINE

The role of pyridoxine in the metabolism of tryptophan has been the subject of study by several investigators. Tissue homogenates from pyridoxine-deficient animals show impaired conversion of *l*-kynurenine to anthranilic and kynurenic acids in vitro, a defect correctible by the addition of pyridoxal phosphate to the preparation.[40] In rats, simple dietary deficiency of pyridoxine has little effect upon the conversion of tryptophan to liver pyridine nucleotides, but if the deficiency is accentuated by the administration of desoxypyridoxine, conversion is definitely inhibited.¶ Numerous metabolites of tryptophan, among them kynurenine, 1,3 hydroxykynurenine, kynurenic acid, and xanthurenic acid, are found in the urine of pyridoxine-deficient animals after the administration of tryptophan. The fact that all of these metabolites retain the carbon atoms of the original alanine side-chain of tryptophan suggests that pyridoxine participates in tryptophan metabolism at the stage at which this side-chain is split from the rest of the molecule.[57]

Sinclair [58] observed that pyridoxine deficiency in rats results in diminished histaminase activity of skin, intestine, and lung and an increase in the dermal reaction to the injection of histamine. He found that administration of antihistaminic

¶ References 55 and 56.

drugs to deficient animals resulted in improvement in skin and mucocutaneous lesions. These findings have been interpreted as suggesting that pyridoxine (pyridoxal phosphate) may act as a coenzyme of histaminase and that the skin lesions of pyridoxine deficiency may be secondary to vascular changes produced by abnormal concentrations of histamine. Binkley and associates [59] have found that the enzymes responsible for the transfer of sulfur from homocysteine to the carbon chain of serine may be markedly activated by the addition of minute amounts of pyridoxal phosphate. Pyridoxine deficiency led to the production of severe anemia in monkeys [60] but not in rats.[61] Lymphopenia occurs in the latter animal and may be accentuated by the administration of desoxypyridoxine.[62] Two of three adult human subjects with acute lymphatic leukemia who received desoxypyridoxine showed a marked drop in the white blood cell count, regression of lymph nodes, and general clinical improvement.[63]

The development of hypertension in rats made deficient in pyridoxine has been reported by Olsen and Martindale.[64] The addition of pyridoxal to the diet of these animals was followed by a gain in weight and significant reduction in blood pressure. Wachstein and Gudaitis # confirmed and extended the previous findings of Sprince and associates [67] that abnormally large amounts of xanthurenic acid are excreted in the urine by pregnant women after the administration of a tryptophan load test. This increase in xanthurenic acid excretion begins near the end of the first trimester of pregnancy and is greater in pregnancy complicated by toxemia than in normal pregnancy. After the daily administration of 25 mg. of pyridoxine hydrochloride, urinary values for xanthurenic acid return to normal. No data regarding blood pressure changes in the patients with toxemia were reported.

Schreiner and associates [68] have observed that a large percentage of patients with seborrheic dermatitis of the sicca type responded well to local application of pyridoxine but not to oral or parenteral administration of the vitamin. These findings suggest the presence of a local metabolic defect in the skin, possibly involving fat metabolism, which may increase the local requirement for pyridoxine.[69]

PANTOTHENIC ACID

The importance of pantothenic acid in metabolic processes is being increasingly recognized. As a constituent of coenzyme A, it participates in a large variety of acetylation reactions.[1] Coenzyme A occupies a key position in both carbohydrate (pyruvate) and fat (acetate) metabolism through the catalysis of citrate formation.[70] This coenzyme appears to have a role in the synthesis of lipids and steroids, in the formation of peptides, and as a transphosphorylating agent in tissues.

In animals, pantothenic acid deficiency is associated with changes in structure and function of the adrenal glands.* Ralli [73] found that large doses of calcium pantothenate influenced certain blood and urinary findings in human subjects exposed to cold stress.

Despite the wealth of information concerning panthothenic acid in animal metabolism, there are no data, as yet, concerning human panthothenic acid requirement or the role of this vitamin in human nutrition.

References 65 and 66.

* References 71 and 72.

LIPOIC ACID

The role of lipoic acid (thioctic acid; protogen) in human and animal nutrition has not yet been established, although this compound possesses certain attributes which may eventually lead to its classification as a vitamin of the B complex. It is essential for the growth of several micro-organisms and functions in the oxidative decarboxylation of α-keto acids. It apparently participates in the oxidation of pyruvate in the form of a molecular conjugate with thiamine pyrophosphate, known as lipothiamide pyrophosphate.† Coenzyme A also participates in this process.[76] It has been proposed that lipoic acid may play a key role in the conversion of light energy into chemical energy in the process of photosynthesis.‡

FOLIC ACID (FOLACIN, PTEROYLGLUTAMIC ACID) AND CITROVORUM FACTOR (FOLINIC ACID)

A recent monograph by Jukes,[79] entitled "B-Vitamins for Blood Formation," should prove a most useful reference for anyone wishing to review the chemistry, functions, and therapeutic efficacy of folic acid and citrovorum factor. Both folic acid and citrovorum factor occur in nature in conjugated forms, which largely accounts for incomplete knowledge of the distribution of these factors in food. Further study is being conducted in this field,[80] and new methods of assay are being developed.[81] When accurate information is available, determination of the human requirement for this vitamin group will become feasible.

Synthetic citrovorum factor, "leucovorin," appears to have half the activity, weight for weight, of the pure natural vitamin.[82] In general, preparations of natural and synthetic citrovorum factor have essentially the same properties.[83] The distribution of folic acid and citrovorum factor in human tissues obtained at autopsy was studied by Girdwood.[84] In two nonanemic subjects the liver and kidneys contained relatively large amounts of citrovorum factor but no folic acid. In one patient with pernicious anemia, who died at the beginning of treatment with vitamin B_{12}, the liver contained appreciable amounts of both citrovorum factor and folic acid.

Factors involved in the conversion of folic acid to citrovorum factor continue to be investigated. Ascorbic acid has been implicated in this conversion,[82] and a role for xanthine oxidase has been postulated.[85] Recently, ascorbic acid has been found to activate the enzyme which liberates citrovorum factor from the conjugated form.§

The role of folic acid in cellular metabolism has been reviewed.[88] One of the functions of citrovorum factor is related to the biochemical transfer of "single carbon" fragments.[79] Verly and associates [89] showed that folic acid, and also leucovorin, stimulated synthesis of the methyl group in choline from methanol. Stekol and co-workers [90] presented data indicating that folic acid or its derivative is involved in the synthesis of both moieties of choline from serine and through serine from glycine.

Vitamin B_{12} is also involved in the synthesis of methyl groups.[91] Dietrich and associates [81] found that rats deficient in folic acid lacked the ability to methylate efficiently and to excrete as N^1-methylnicotinamide (N^1-Me) large doses of nicotin-

† References 74 and 75.

‡ References 77 and 78.

§ References 86 and 87.

amide which were administered. Folic acid in 10 γ doses produced a significant increase in the output of N^1-Me, while leucovorin in doses of 10 and 100 γ was without effect. It was suggested that folic acid may be the immediate precursor of the physiologic activator or coenzyme involved in the methylation of nicotinamide or in the synthesis of methyl groups. The relation of vitamin B_{12} to methylation was also discussed; its role appears to be more indirect and complex. It seems likely that folic acid and vitamin B_{12} will be found to be related to metabolism of methyl groups in man.

A relationship between folic acid and coenzyme A has been reported by Popp and Totter.[92] Bertolani and Lorenzini [93] observed that folic acid accelerated the appearance of signs of thiamine deficiency in pigeons on diets deficient in thiamine. Signs of deficiency regressed following administration of thiamine, a finding at variance with those of previous studies in which experimental beriberi, aggravated by folic acid administration, was reported to be irreversible.

Lee and associates [94] produced hypertension and renal lesions in rats by intraperitoneal injections of folic acid for a relatively long period. It was suggested that a renal factor, comparable to that produced by interference with renal blood flow, might be involved. Other mechanisms suggested were interference with renal or other enzyme systems that normally inactivate hypertensive factors or acceleration of transmethylation to a degree sufficient to induce deficiency of accessible methyl groups.

Metabolic relationship between ascorbic acid and folic acid has been a subject of considerable interest. May and associates || have conducted extensive studies of the megaloblastic anemia which occurs as a complication of scurvy in monkeys fed milk diets. In such monkeys many phases of folic acid metabolism appear to proceed normally. Conversion of folic acid to citrovorum factor seemed less efficient than in normal animals, but ascorbic acid was not essential for this conversion. Megaloblastosis of the bone marrow did not occur unless the level of free citrovorum factor in the liver was low (total folic acid was also low). Urinary excretion of folic acid did not prove to be a practical index of tissue stores. Folic acid and *l*-ascorbic acid produced reversion of the bone marrow to normal and caused accumulation of folic acid and citrovorum factor in the liver. Citrovorum factor was effective in smaller doses than folic acid in restoration of the marrow to normal. Vitamin B_{12} neither prevented nor improved megaloblastic anemia in scorbutic monkeys. Folic acid administration will prevent megaloblastosis during scurvy, but a normoblastic anemia may develop which appears to be due to hemorrhagic phenomena or defective absorption of iron. It was suggested that folic acid deficiency in scurvy is due to an increased requirement of this vitamin. No direct specific function of ascorbic acid in hemopoiesis was detected.

Gabuzda and associates [99] studied excretion of folic acid and citrovorum factor in patients with scurvy. Administration of folic acid was followed by only slight increases in citrovorum factor excretion until adequate therapy with ascorbic acid had been administered. The authors concluded that ascorbic acid greatly facilitates conversion of folic acid to citrovorum factor. In another study,[100] citrovorum factor failed to influence either tyrosyl excretion or abnormal capillary fragility in clinical scurvy.

|| References 95-98.

Several studies of the relationship of folic acid to experimentally induced anemia in animals have been reported In anemia produced in male mice by intraperitoneal injection of phenylhydrazine hydrochloride, both folic acid and vitamin B_{12} were found essential for stimulation of optimum hemopoiesis.[101] Citrovorum factor was effective in large amounts; vitamin B_{12}, in small quantities. A macrocytic anemia was induced in swine, which responded rapidly and completely to both folic acid and vitamin B_{12}.[102] Only partial remission followed administration of vitamin B_{12} alone, while remission, with subsequent relapse, followed therapy with folic acid alone. In rats, macrocytic anemia developed when a small intestinal cul-de-sac, with stagnation of contents, was produced in the upper small intestine.[103] The marrow was not megaloblastic, and there was evidence of a hemolytic element. Hemopoietic response followed administration of folic acid or chlortetracycline (Aureomycin) but not vitamin B_{12}. A megaloblastic anemia was induced in monkeys by the injection of turpentine intramuscularly.[104] A low level of folic acid compounds was observed in the livers of these animals. Administration of folic acid eliminated megaloblastosis, whereas vitamin B_{12} and ascorbic acid were ineffective.

In a study of the utilization of folic acid in normal subjects, Spray [105] found that oral administration of yeast extract, containing folic acid largely in conjugated form, led to a less marked increase in folic acid in plasma and in urine than did administration of pure folic acid. These findings suggest that only small proportions of folic acid compounds in food are absorbed. Spray and Witts [106] reported that oral administration of 1 mg. of folic acid was followed by a smaller increase in plasma level and urinary excretion in patients with untreated pernicious anemia than in normal subjects. After therapy with liver extract or vitamin B_{12} patients with pernicious anemia showed a normal response. These results were interpreted as indicating impaired absorption or increased utilization of folic acid in pernicious anemia in relapse. In a subsequent study, excretion of both folic acid and citrovorum factor after a 5 mg. dose of folic acid was found to be lower in patients with pernicious anemia in relapse than in normal subjects.[107] These results indicate that there may be either deficiency of conversion of folic acid to citrovorum factor or an increased avidity of the tissues for citrovorum factor in pernicious anemia in relapse. Girdwood [108] compared folic acid excretion after oral and parenteral administration of doses of 5 mg. An excretion of less than 1.5 mg. after an oral dose was considered indicative of severe tissue depletion or malabsorption. If tissues had been loaded with folic acid prior to testing, low excretion after an oral as compared with a parenteral dose was indicative of malabsorption. The "differential folic acid excretion test" was suggested as of particular value where steatorrhea was not obvious or where there was refractory megaloblastic anemia, refractory iron deficiency anemia, or unexplained glossitis.

The role of "leucovorin" in nutrition, leukemia, and the megaloblastic anemias was the subject of a recent review,[82] and the use of folic acid in megaloblastic anemias refractory to vitamin B_{12} has been summarized.[109] Diez-Rivas and associates [110] reported satisfactory hematologic and clinical response in six patients with tropical sprue who were given oral therapy of folic acid (1.67 γ) and vitamin B_{12} (25 γ) daily. They suggested that folic acid "potentiates the hematopoietic effect of orally administered vitamin B_{12}" and that in sprue there is deficiency of both

vitamins. Romero and associates [111] reported that parenteral administration of citrovorum factor to nine patients with sprue was followed by clinical and hematologic improvement.

Thompson [112] demonstrated that folic acid has a direct maturing effect on cultures of megaloblasts obtained from patients with pernicious anemia, in confirmation of studies of Lajtha.[113] No effect was observed with liver extracts, vitamin B_{12}, or thymine. Horrigan and associates [114] previously demonstrated a maturation effect in patients with pernicious anemia when vitamin B_{12} was injected into the bone marrow of one iliac crest, but there was no effect when folic acid was so injected. The action of these substances in marrow culture may differ from their action in vivo. Relationships between vitamin B_{12}, folic acid, and citrovorum factor were reviewed by Girdwood.[115]

The metabolism of folic acid in patients with leukemia was studied by Swendseid and associates.[116] Average folic acid excretion following test doses of the vitamin was significantly lower in patients with leukemia than in normal subjects, although wide variations were observed. Depletion of body stores of folic acid was greater in chronic leukemia, suggesting that the varying clinical results obtained with therapy with folic acid antagonists may be related to degree of nutritional depletion. Other data indicated that 4-aminopteroylglutamic acid (Aminopterin) metabolically displaces folic acid.

The effect of folic acid deficiency on reproduction in rats was reported by Nelson and associates.[117] Fetal resorption or congenital abnormalities were produced in many animals, the effect varying with the time after breeding at which deficiency was induced. From the standpoint of human nutrition, it is of interest that administration of a folic acid antagonist, 4-aminopteroylglutamic acid, produced therapeutic abortion in 10 of 12 patients who were less than three months pregnant.[118]

VITAMIN B_{12}

The discovery and identification of vitamin B_{12} have been reviewed by Smith,[119] who, with his associates, has also reported further studies of the characteristics of some of the related cobalamin compounds.¶ The substance initially identified as "vitamin B_{12d}" was found to be identical with vitamin B_{12b}, the original characterization as a separate compound being attributable to an anomaly in the technique of partition chromatography. The production of radioactive isotopically-labeled vitamin B_{12} by neutron irradiation [124] and by biosynthesis [125] has also been reported by this group. Lewis and co-workers # reported the isolation from rat feces of a substance, designated "vitamin B_{12f}," which is similar to, and possibly identical with, pseudovitamin B_{12}, which is obtained from rumen contents. It has been found to be active in promoting the growth of Lactobacillus leichmannii and of chicks, but it is ineffectual in promoting growth in rats.

Studies in various experimental animals maintained on purified diets have demonstrated that the administration of vitamin B_{12} results in increased food intake and enhancement of growth rate.* The growth-promoting effect may be increased

¶ References 120-123.

References 126 and 127.

* References 128-131.

somewhat if antibiotics are administered simultaneously with the vitamin,† and it is apparently due to alterations in the metabolism of carbohydrate and fat rather than in that of protein.‡ Other compounds structurally related to vitamin B_{12} possess the ability to promote growth in vitamin-B_{12}-deficient animals to varying degrees but do not increase tissue stores of the vitamin.[132] The total nucleic acid content of the livers of rats maintained on diets deficient in vitamin B_{12} is less than that observed in normal animals, although the content per cell is the same in both groups. The rate of incorporation of isotopically-labeled aminoacetic acid (glycine) into the purines of ribonucleic acid is also decreased in the vitamin-deficient animals. These observations suggest that in vitamin B_{12} deficiency there is a reduction in the rate of cell division in the liver, brought about by a decrease in the rate of nucleic acid synthesis.[133]

Studies of the effect of administration of vitamin B_{12} to children with growth failure, in Ohio [134] and the Aleutian Islands,[135] have shown an increase in growth rate, as measured by the Wetzel grid method, when the diet was supplemented with 6 to 10 γ of the vitamin daily. However, Finberg and Chow [136] observed no effect upon rate of growth or hematologic status of 54 premature infants whose diets were supplemented with 30 γ of the vitamin daily. The administration of 5 γ of vitamin B_{12} daily for periods of four or eight weeks did not accelerate growth of newborn infants.[137] Additional studies seem indicated before vitamin B_{12} is generally accepted as a growth factor in human nutrition.[138]

Studies by Meites § suggest that marked increases in levels of thyroid hormone, estrogen, or cortisone in rats can increase requirements for vitamin B_{12} and, possibly, requirements for other dietary factors. The addition of vitamin B_{12} or chlortetracycline to the diets of animals receiving these hormones can partially or completely prevent manifestations of dietary deficiency of vitamin B_{12}. The characteristic action of the hormones was, in general, not altered by such dietary supplementation, but in the case of cortisone atrophy of the thymus was prevented. D'Iorio and Plant,[141] however, found that the reduction in epinephrine content of the adrenals which follows the addition of thyrotoxic materials to the diet of rats was partially prevented by the administration of vitamin B_{12}. They suggested that the vitamin might have a function in the formation of the methyl groups of epinephrine. Other observers have found that vitamin B_{12} prevents hypertrophy of the spleen which follows the administration of thyroid to rats on a casein-sucrose diet.[142] Gershberg and Ralli [143] observed that vitamin B_{12} exerted a distinct lipotropic effect upon the livers of rats fed a diet low in protein and high in fat but did not prevent an increase in the cholesterol content of the adrenal glands.

Studies of Arnstein and Neuberger [144] indicated that vitamin B_{12} had little or no effect upon the conversion of formate or the β carbon atom of serine to choline methyl groups in the rat. The vitamin does participate in the formation of N^1-methylnicotinamide, an excretion derivative of nicotinamide.[145] Dryden and associates,[146] found that the high mortality among offspring of rats fed purified casein rations could be prevented in most, but not all, instances by addition of vitamin B_{12} to the diet.

† References 128 and 129.

‡ References 128-131.

§ References 139 and 140.

A chemical method for the determination of vitamin B_{12}, based upon the difference between the absorption spectra of the vitamin and its dicyanide complex, has been found suitable for the assay of this substance in solutions containing relatively high concentrations, such as fermentation broths and crude concentrates.[147] The difficulties inherent in microbiological techniques used for the quantitative measurement of vitamin B_{12} were reviewed by Ford,[148] who emphasized the necessity for precise definition of the conditions under which such assays are performed. An excellent review of the role of vitamin B_{12}, folic acid, and other members of the vitamin B complex in the metabolism of micro-organisms was presented by Wood.[149]

Studies of the uptake of radioactive vitamin B_{12} by a number of micro-organisms revealed that the degree of incorporation of radioactivity into bacterial cells bore no relationship to the requirement of the organism for the vitamin to sustain growth.[150] Radioactive tracer techniques were employed by Oginsky [151] to study the uptake of vitamin B_{12} by normal and mutant strains of Escherichia coli. Davis [152] found that those mutants of E. coli which show a growth response to vitamin B_{12} or methionine respond in similar fashion to pseudovitamins B_{12} and B_{12b}. E. coli was also employed by Dubnoff [153] to study the role of vitamin B_{12} in methionine synthesis.

Euglena gracilis, a green alga, is another organism which requires vitamin B_{12} for growth and which has been adapted to microbiological assay techniques. Studies of the specificity of vitamin B_{12} for this organism have revealed that it is capable of being stimulated by other substances related to the vitamin.[154] An oxidation product of vitamin B_{12} does not inhibit growth of this organism when the medium contains the vitamin in concentrations which normally support full growth.[155] Ross [156] found that vitamin B_{12} in serum and urine could be assayed with Euglena and that the vitamin was present in serum in both free and protein-bound form, the latter predominating. It was found necessary to heat serum to 100 C. for one-half hour before the bound form could be measured. Employing this technique, Mollin and Ross [157] studied the vitamin B_{12} concentrations of serum and urine of normal subjects and of patients with a variety of diseases. Patients with megaloblastic anemias responsive to vitamin B_{12} therapy had significantly lower concentrations of the vitamin in their serum than did normal subjects. Patients with similar anemias, but refractory to vitamin B_{12} therapy, had normal concentrations of the vitamin in their serum. Urinary excretion of the vitamin was considerably less in seven of eight pataients with pernicious anemia than in normal subjects.

L. leichmannii has also been used for the assay of vitamin B_{12}, but it is somewhat less sensitive an organism than Euglena. It has been proposed that resting cells of this organism be used to concentrate vitamin B_{12} from dilute solutions, following which the vitamin might be eluted and assayed more accurately.[158] Rosenthal and Sarett [159] described a method for determination of the total vitamin B_{12} activity of human serum which employs this Lactobacillus as the test organism. Results obtained with this technique compare well with those obtained with Euglena. The method has been applied by Unglaub and Rosenthal [160] to the study of serum vitamin B_{12} levels in normal subjects and in patients with megaloblastic anemias before and after oral or parenteral administration of the vitamin. Girdwood [84] reported that liver, kidney, spleen, and brain of nonanemic human subjects, obtained at autopsy, con-

tained larger amounts of vitamin B_{12} than other organs of the body. None of the vitamin could be demonstrated in the tissues of patients who died with untreated pernicious anemia.

The urinary excretion of vitamin B_{12} following intramuscular injection of the vitamin has been studied by Sokoloff and associates [161] and by Lang and his co-workers.[162] In general, urinary excretion of vitamin B_{12} increases with increasing dosage.

The factors involved in the pathogenesis of the megaloblastic anemias and the role of vitamin B_{12} in therapy of these conditions have been reviewed by Ungley [163] and by Clough.[164] The use of vitamin B_{12} in clinical practice has been evaluated by Finch.[165] Girdwood || has discussed vitamin B_{12} in its relationship to folic and folinic acids.

The mechanism of absorption of vitamin B_{12} from the gastrointestinal tract and the role of "intrinsic factor" in the etiology of pernicious anemia have been subjects of increasing interest among investigators. Heinle and his associates [167] administered vitamin B_{12} labeled with radioactive Co^{60}, orally, to normal subjects and to patients with pernicious anemia. The amount of radioactivity which appeared in the feces was greater in patients with pernicious anemia than in normal subjects but could be decreased by administration of a source of intrinsic factor in conjunction with the labeled vitamin.

Lajtha [168] and Thompson [112] reported studies of the effect of various agents upon the maturation of megaloblasts in cultures of human bone marrow. The number of these cells in the marrow preparations definitely decreased when they were incubated in normal serum but not when they were incubated in serum from patients with pernicious anemia in relapse. Their results also indicated the presence of a factor in human serum which is necessary for the "megaloblast ripening" action of vitamin B_{12}, as the crystalline vitamin alone was without effect upon the in vitro preparations. In addition, Lajtha's ¶ observations suggest that there may be a factor in the serum of patients with pernicious anemia which inhibits the "megaloblast ripening" action of vitamin B_{12} in vitro. Feinmann and associates,[169] employing a similar technique, were unable to demonstrate the presence of any such inhibitory factor. Horrigan and Heinle [170] have observed a patient with a macrocytic anemia in whom remissions could be induced by either parenteral administration of vitamin B_{12} or the infusion of normal plasma. Remission was not maintained by frequent injections of the vitamin. Studies with radioactive vitamin B_{12} suggested a defect in binding of the vitamin by serum. It must be noted that free hydrochloric acid was present in the gastric secretion of this subject and the bone marrow was not megaloblastic.

A mucoprotein, derived from human gastric mucosa, has been demonstrated to potentiate the action of vitamin B_{12}, administered orally, in patients with pernicious anemia.# It was suggested that estimation of the amount of this substance present in gastric secretions might be helpful in determining which patients with megaloblastic anemia might be expected to respond to oral therapy with vitamin B_{12}. Landböe-Christensen and co-workers [173] compared the intrinsic factor activity of dessicated defatted human gastric fundus, duodenum, and jejunum and found the

|| References 115 and 166.
¶ References 113 and 168.
# References 171 and 172.

greatest activity to be present in the fundus preparation. Studies of the effects of heat upon the microbiological and antianemic properties of human gastric juice mixed with vitamin B_{12} suggest that the factor in the gastric juice which binds the vitamin, and makes it unavailable for the growth of L. leichmannii, is not identical with the intrinsic factor of Castle.[174] Other microbiological studies with intrinsic factor preparations have demonstrated that the addition of these substances to vitamin B_{12} prevents absorption of the vitamin by bacteria which are commonly present in the intestinal tract.* It does not seem likely that this effect can explain the action of intrinsic factor in the human subject, since small doses of the vitamin introduced into washed isolated loops of intestine or administered by mouth after reduction of the intestinal flora by antibiotics are without hemopoietic effect.[177]

Chemical studies by Hausmann and Mulli [178] have indicated the existence in mammalian liver of a vitamin B_{12}-peptide conjugate, the hemopoietic effect of which is strikingly reduced in comparison with that of the free vitamin.

In the past year, clinical studies have yielded further evidence that parenteral administration of vitamin B_{12} is completely effective in the treatment of pernicious anemia.† Studies of Murphy and Howard [182] suggest that 1.3 γ of the crystalline vitamin is equivalent to 1 U. S. P. unit of liver extract. When therapy with vitamin B_{12} is begun in patients with pernicious anemia, the earliest biochemical alteration observed is a rapid decrease in the excretion of phosphorus in the urine, followed by an increase, to greater than normal levels, during the peak of the reticulocyte response and then a gradual return to normal.[183] These changes may be related to changes in nucleoprotein metabolism which accompany the alteration of a megaloblastic to a normoblastic bone marrow. Other biochemical changes observed during early remission induced by the vitamin include the production of positive nitrogen balance of as much as 6.0 gm. daily and an increase in urinary excretion of uric acid.

The use of single massive doses of 1,000 γ of vitamin B_{12}, intramuscularly, in the therapy of pernicious anemia has been studied by Walker and Hunter.[184] On the basis of urinary excretion studies, it would seem that this is not a desirable regimen, since almost all of such a large dose would be promptly excreted in the urine and little more retention in the body could be anticipated than would occur after the administration of smaller doses. Conley and associates [185] reported results of prolonged therapy of pernicious anemia with vitamin B_{12}. Maximum neurologic improvement in patients with subacute combined degeneration of the spinal cord was obtained with dosages which were sufficient for maximum blood regeneration, and such improvement was not accelerated by the use of massive doses. Several patients were treated with intramuscular injections of 150 γ of the vitamin at four- to five-month intervals. Results were reported to be as satisfactory in this group as in another group adequately maintained on 45 γ every six weeks. The crystalline vitamin was also found to be effective when given orally in doses approximately 100 times as large as the effective parenteral dose, as has been noted by Ungley [177] and others.

Sanneman and Beard [186] administered a combination of folic acid and vitamin B_{12}, parenterally, to eight patients with pernicious anemia whose blood picture

* References 175 and 176.

† References 179-181.

had not been maintained satisfactorily with vitamin B_{12} alone. No significant change in hematologic status was observed in these patients. Reisner and Weiner [187] noted "enhanced hemopoietic response of some pernicious anemia patients to combined oral doses of folic acid and vitamin B_{12}, in themselves suboptimal." In corroboration of earlier findings, additional cases of megaloblastic anemia of pregnancy, nontropical sprue, and nutritional megaloblastic anemia have been reported, in which response to folic acid therapy has been observed after the failure of vitamin B_{12} to produce a hemopoietic response.‡

Suárez and co-workers [190] reported that single oral doses of 100, 500, and 1,000 γ of vitamin B_{12} were ineffective in the treatment of tropical sprue but that good responses were obtained with doses of 3,000 or 5,000 γ. They observed no effect when the vitamin was given sublingually in daily doses of 25 γ. Cohen, Meyer, and Fadem [191] reported a case of sprue, refractory to large oral and parenteral doses of vitamin B_{12}, which responded to therapy with folic acid. Diez-Rivas and his associates [110] found that the oral administration of vitamin B_{12} and folic acid together, in amounts ineffective when either was given alone, produced a satisfactory clinical and hematological remission in each of six cases of tropical sprue. They concluded that a deficiency of both substances occurs in this condition.

Campbell and Pruitt [192] reported that oral administration of 30 γ of vitamin B_{12} to patients with virus hepatitis during the first five days of their hospitalization resulted in more rapid return of appetite, reduction in size of the liver, shorter duration of clinical disease, and fewer relapses than in an untreated control group. It has been reported that urinary porphyrin excretion decreases to normal levels after administration of vitamin B_{12} to patients with lead poisoning.[193] The vitamin is not effective in the treatment of acute leukemia when given intramuscularly in doses of 1,000 γ daily.[194] Concentrates containing vitamin B_{12}, prepared from cultures of Streptomyces griseus, may produce sensitivity reactions in some patients.[195] Positive skin sensitivity tests were found to be twice as common in persons who had previously received antibiotics as in those who had received none. The possibility exists that such crude preparations of the vitamin may sensitize patients to antibiotics derived from Streptomyces. No untoward reactions have been reported following administration of the pure crystalline vitamin.

ASCORBIC ACID

The role of ascorbic acid in metabolism is being gradually elucidated. Recent studies in guinea pigs indicate that catalytic amounts of ascorbic acid are required for oxidation of tyrosine by way of *p*-hydroxyphenylpyruvic acid.§ The requirement is not specific, as isoascorbic and glucoascorbic acids, as well as reductone, are also active. Hydroxyphenyl compounds (metabolites of phenylalanine and tyrosine) are known to be excreted in abnormal amounts in scurvy. Aterman and associates [198] suggest that urinary excretion of *p*-hydroxyphenylacetic acid "may be regarded as an early indication of suboptimal intake of ascorbic acid." Steele and associates [199] found no change in urinary excretion of hydroxyphenyl compounds in subjects maintained for 78 days on a diet which supplied only 7

‡ References 188 and 189.

§ References 196 and 197.

mg. of ascorbic acid and which was supplemented with 10 gm. of *l*-tyrosine daily. Davey and co-workers [200] found no indication of disturbed metabolism of aromatic amino acids in four subjects whose intake of ascorbic acid was 25 mg. daily for 52 days. In the studies of Steele and Davey and their respective associates,‖ serum ascorbic acid declined to about 0.3 mg. per 100 cc., which indicates that severe depletion of ascorbic acid stores was not attained. In experimental scurvy ascorbic acid is essentially absent from the serum for many weeks before signs of deficiency appear.

Possible relationships of ascorbic acid to adrenal cortex function have been widely investigated.[201] A number of interesting findings have been reported in the past year. Becker and associates [202] studied the relationship of ascorbic acid to steroid synthesis. They found that, although 1-C^{14}-labeled ascorbic acid was not appreciably incorporated into cholesterol, the vitamin exerted a marked effect on the conversion of acetate-1-C^{14} to cholesterol and other steroids. Severely scorbutic guinea pigs, in comparison with normal animals fed ad libitum, incorporated six times as much C^{14} from acetate-1-C^{14} into cholesterol of the adrenal glands.

Banerjee and Deb [203] found that 17-ketosteroid excretion was slightly reduced in guinea pigs with scurvy. In monkeys on scorbutigenic diets, Stewart and associates [204] observed a decrease in 17-ketosteroid excretion followed by a rise as manifestations of scurvy appeared. Injection of adrenocorticotropic hormone was followed by an increase in 17-ketosteroids in the urine comparable to that found in normal animals. These findings suggest that function of the adrenal cortex was unimpaired. In human studies, Kayahan [205] reported that administration of large amounts of ascorbic acid (sufficient to saturate the body with this vitamin) was followed by increased excretion of 11-oxysteroids and decreased excretion of 17-ketosteroids. Since this effect differs from that of corticotropin, it was considered not to be mediated by the pituitary gland but rather to result from direct action on the adrenal cortex. In studies on hypophysectomized rats, Dugal and Thérien [206] found that ascorbic acid alone had no effect on regeneration of the adrenal cortex but potentiated the effect of corticotropin on this process. Bacchus and associates ¶ noted that ascorbic acid enhanced the gluconeogenic activity of cortisone in adrenalectomized mice and prolonged the leucocytic effects of injection of cortisone or corticotropin in rats, adrenalectomized or adrenal-demedullated, respectively. The authors also stated that ascorbic acid prevents the alarm reaction of acute stress in experimental animals, whether induced by trauma or epinephrine. They postulated that ascorbic acid blocks the activation of the pituitary-adrenal axis by inhibiting, in some manner, the activation of the pituitary. Ascorbic acid may decrease inactivation of cortical hormones, which, in turn, depress release of corticotropin by the pituitary.

Pirani and associates [209] noted severe regressive changes in the scar tissue of healed wounds when scurvy was induced, subsequently, in guinea pigs. They concluded that adequate ascorbic acid is required not only for normal healing but for the maintenance of scar tissue which has formed in healed wounds over a period

‖ References 199 and 200.

¶ References 207 and 208.

of many weeks. Robertson # reported that the collagen concentration of healed wounds in new subcutaneous tissue was not lowered when guinea pigs were made scorbutic. It would thus appear that the findings of Pirani cannot be explained by a decrease in collagen.

Daft and Schwarz [212] have prevented or delayed the appearance of signs of pantothenic acid or riboflavin deficiency in rats when the diet contained 2% of ascorbic acid. In a diet free of thiamine, 5% ascorbic acid had a similar protective effect.

Lee and Holze [213] found that the ability of the scorbutic guinea pig to withstand hemorrhage was impaired. Plasma from the deficient animal was devoid of vasoexcitor material (VEM), as determined by bioassay with the rat mesoappendix test. Akers and Lee [214] recently obtained evidence that the absence of VEM can be attributed to impaired renal production. This failure of VEM formation is not limited to a single dietary deficiency, as rats on a "cirrhotic diet" do not elaborate this factor.

Davis and Oester [215] reported that administration of ascorbic acid lowered the incidence of experimental aortic sclerosis induced by thyroxine and epinephrine injections in rabbits. Wexler [216] found a marked depletion of ascorbic acid in the adrenal gland of rats after total body irradiation with x-ray.

Relationships between ascorbic acid and folic acid have continued to receive attention and are discussed in the section dealing with the latter vitamin.

The existence of bound ascorbic acid in plant tissues has been known since 1938.[217] No satisfactory procedure for determination of bound ascorbic acid in animal tissues has been available. Recently, Sumerwell and Sealock [218] have devised a procedure which appears to give reproducible and reliable values for liver tissue. Appreciable amounts of ascorbic acid in bound form were detected, leading the authors to question the accuracy of values accepted at present for ascorbic acid content of foods.

A complete report of the experiments on vitamin C deprivation in man, which were conducted by a subcommittee of the British Medical Research Council in 1944-1945, has been published.[219] The report contains a wealth of interesting clinical and laboratory data and a number of excellent black-and-white and color photographs. Merrow and associates [220] studied serum levels and nutrient intake of ascorbic acid in Vermont school children. Only in selected, small groups of children was there a demonstrable relationship between serum levels and nutrient intake. Getz and associates,[221] in a study designed to correlate previous nutritional status with the appearance of tuberculosis, found that in the 28 presumably nontuberculous persons who subsequently developed active tuberculosis values for ascorbic acid concentration of the blood were all at the lower range of values found in the group of 1,100 persons who were under investigation. The study needs confirmation, since proof of absence of tuberculosis in these 28 subjects was not unequivocal. Kirk and Chieffi,[222] in a study of 142 patients aged 40 to 93, reported a decline in ascorbic acid concentration in the blood with age. Lowry and associates [223] found that administration of 1 gm. of ascorbic acid daily to four patients for three months was without any progressive change in serum or leucocyte level, tolerance curve, or urinary excretion. No harmful effects were observed.

References 210 and 211.

VITAMIN D

The studies of Zetterström and Ljundgren suggesting an enzymatic role of vitamin D in the activation of alkaline phosphatase have been reviewed.[224] Boyer [225] has pointed out alternative explanations for the results reported by these authors. Räihä and Forsander [226] have found that the administration of vitamin D to human subjects receiving small daily doses of thiamine intramuscularly results in increased cocarboxylase concentration in the blood, presumably via the accelerated phosphorylation of thiamine. Harrison and Harrison [227] investigated changes in citrate concentration in serum and urine of 10 rachitic infants before and after the administration of vitamin D. Initial levels of serum citrate were found to be significantly lower than those of normal infants, and they increased to within or above normal limits after therapy. Improvement in bone lesions was always accompanied by a rise in serum citrate. Increases in urinary citrate excretion after therapy were observed in three infants. Similar changes in urinary citrate excretion after vitamin D administration have been observed in rats.[228] The mechanism of these effects is not known, and further studies would seem desirable.[229]

VITAMIN E

Techniques for the separation and simultaneous analysis of tocopherol mixtures by paper chromatography have been developed by Brown * and may prove useful in analysis of tissue tocopherols. Farber and associates [232] reported a simple macro method for the determination of plasma tocopherols, which requires only 1 to 3 ml. of plasma or serum. Klatskin and Molander † applied a molecular distillation technique to the determination of total tocopherols in feces. They found the daily excretion of tocopherols in normal subjects to be 21.62 ± 2.90 mg., or an average of 64.4% of the daily intake. Their studies of the absorption and excretion of tocopherols in Laennec's cirrhosis have been discussed in a previous review.[38] The physiological role of vitamin E has been reviewed by Mattill.[235]

LaMotte and associates [236] reported that α-tocopherol acetate appears to be of little value in the prophylaxis or therapy of retrolental fibroplasia in premature infants. Welsh [237] reported that vitamin E, combined with large doses of pantothenate, produced favorable results in patients with discoid or subacute disseminated lupus erythematosus. Mastboom and Sikkel [238] did not find vitamin E to be of value in the management of toxemia of pregnancy. In 46 cases of Dupuytren's contracture, 100 mg. of the vitamin given twice daily for a period of three months was reported to be without beneficial effect.[239] Additional cases of peripheral venous thrombosis treated with alpha tocopherol have been reported.[240]

VITAMIN K

Vitamin K_1 has been found effective in the treatment of hypoprothrombinemia induced by bishydroxycoumarin ‡ or ethyl biscoumacetate (Tromexan).[243] Animal experiments, utilizing menadione labeled with radioactive C^{14} in the methyl group, demonstrated rapid excretion of the vitamin in the urine after injection, no significant storage in any tissue, and no radioactivity in the prothrombin fraction of the

* References 230 and 231.

† References 233 and 234.

‡ References 241 and 242.

plasma.[244] The authors suggest that vitamin K activity is dependent directly upon blood levels of the vitamin. Vitamin K, given orally in doses of 10 mg. three times daily, did not affect the course of acute viral hepatitis in 178 patients observed by Mossberg.[245] An increase in blood prothrombin levels was obtained in cases in which the disease process was severe, an effect similar to that noted in patients with obstructive jaundice. This suggests that poor absorption of vitamin K is responsible for decreased prothrombin concentration in hepatitis.

MISCELLANEOUS

Sarett [246] studied the urinary excretion of various vitamins of the B complex, or their metabolites, before and after oral administration of streptomycin to human subjects in doses which produced partial or complete inhibition of growth of intestinal bacteria. Results of this study indicate that intestinal bacteria can modify the amount of some of the B vitamins available in the diet. The conversion of tryptophan to nicotinic acid compounds was not affected, supporting the view that this conversion process does not take place in the bowel. Vitamin B complex deficiency syndromes have been noted following prolonged oral administration of some of the newer antibiotics.[247] The feeding of large amounts of methylcellulose was without effect upon the absorption of thiamine or vitamin A administered orally to rats.[248]

Rats made deficient in thiamine, pantothenic acid, biotin, or choline exhibit no specific alteration in lipogenesis as determined by the rate of incorporation of C^{14}-labled acetate into fatty acids and cholesterol.[249] Wertman and associates [250] studied the effect of riboflavin and folic acid deficiencies upon the formation of complement-fixing murine typhus antibodies. The relationship of vitamins to antibody formation was the subject of a review by Axelrod.§

Ershoff ‖ has observed decreased resistance to cold stress in rats made deficient in riboflavin or in vitamin A. This effect, however, may have been influenced by reduction in caloric intake, which was observed in the deficient animals. The role of vitamins and other nutrients in adaptation to stress has been discussed by Goodhart and Jolliffe.[255]

The majority of data concerning the dietary patterns of pregnant women have been derived from predominantly urban population groups. Jeans and associates [256] have studied a large group in a rural section and have pointed out the contribution of enriched bread in furnishing thiamine and riboflavin. Burch and her associates [257] conducted a nutritional resurvey in the Philippines and observed a marked increase in blood and urinary thiamine levels, attributable to the use of enriched rice. Their findings also indicated continued inadequacy of dietary riboflavin and ascorbic acid, as well as of calcium.

§ References 251 and 252.

‖ References 253 and 254.

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SURVEY OF THE 1953 HEMATOLOGY LITERATURE

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This review was supported in part by research grants from the National Cancer Institute of the National Institutes of Health, Public Health Service (C-1576).

Mrs. Helen Chrysler; Mr. James G. Diller; Drs. John H. Wallace, Matthew C. Dodd, Howard M. Sigal, Howard R. Barton, Alex E. Krill, Alvin S. Segel, Joseph D. French, and David Foxman; Mr. Allan Salant; Miss Ada Carver; Mrs. Carolyn Mabry; Mrs. Felicitas Goodman; Mr. William Fredrick Shepard; Mrs. Rosamond Sigal, and Miss Tonita Carter assisted in the preparation of this paper.

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IN THE past decade hematology has developed from groping, awkward adolescence into young adulthood. Developments that have produced this maturity have been amazing to even the most conservative. The entree to this new era was the recognition, isolation, and synthesis of folic acid. This development was responsible for more extensive investigation of the macrocytic anemias and, later, for the discovery of vitamin B_{12}. Biochemists produced analogues of folic acid, which opened new approaches in chemotherapy of the leukemias and lymphomas. Immunohematology has been broadened from the narrow confines of the blood bank to explain many of the enigmas associated with hemolytic anemias and idiopathic thrombocytopenic purpuras. Formerly, the effects of irradiation on the hemopoietic system were primarily the interest of the rare investigator; the atomic and hydrogen bombs have broadened this interest to include the man in the street. Many of the reports made in the 1953 literature reflect the advances that have been made in the past decade.

There is no satisfactory classification for a discussion of this type, owing to overlapping of specific subjects. There are six general categories: special topics, red blood cells, white blood cells, platelets, reticuloendothelial system, and neoplastic blood diseases. The increasing interest in autoimmune blood diseases resulted in an expanded section on immunology, under Special Topics. The polycythemias and leukemias have been taken from their respective sections and included under Neoplastic Blood Diseases, to facilitate discussion. Again, coagulation has been confined to those phases associated with platelet influences.

The literature is progressively mounting. Approximately 1,100 articles are included in this survey. Every attempt has been made to accomplish a comprehensive coverage of the literature; however, the incompleteness of such an endeavor is inevitable. A certain element of discrimination has been attempted; however, editorializing has been kept to the minimum. Liberal use is made of other reviews, editorials, and abstracts of proceedings of recent meetings. There is some overlapping into the preceding and succeeding years.

SPECIAL TOPICS

Techniques

Red Blood Cell Techniques.—Advantages and disadvantages of the photoelectric erythrocyte count have been reviewed,* and an improved method was described by Guiard.[1] A new photomicrographic technique has been used for the enumeration of total red cell counts and Ashby counts without introduction of any additional source of error. Advantages of this technique include the following: 1. A larger number of counts may be made in one laboratory session. 2. The photographs can be made rapidly. 3. Counting may be done at the convenience of the technician. 4. There is a permanent record of each count. The chief disadvantage is that counts cannot be made for an hour after photography.†

A new diluting fluid for counting erythrocytes simultaneously stains the cells and makes them lie flat within a single focal plane.[6] A red-cell-labeling technique using radioactive phosphorus (P^{32}) allows quantitative measurement of local concentrations of red blood cells per unit volume of living tissue at any given moment.[7]

Interesting applications of optical techniques include use of the electrohemoscope for the determination of erythrocyte osmotic resistance,[8] the polarizing microscope for measuring thin spherical birefringent membranes (as in the red cell ghost),[9] the phase-contrast microscope for refractometry studies on red cells,[10] and the compound microscope for measuring violet light (in the Soret band) absorption of heme in living cells.[11]

Techniques of study of red blood cell destruction are considered under the Hemolytic Anemias.

White Blood Cell Techniques.—The direct enumeration of basophilic leucocytes in the counting chamber has been accomplished by employing a solution of saponin in 20% ethyl alcohol (ethanol) as a diluent and toluidine blue as a metachromatic stain.[12] A modification of Ehrlich's hematoxylin and eosin technique allows specific staining of eosinophile granules, leaving the neutrophilic granules practically

* References 1-3.

† References 4 and 5.

unstained.[13] Comparative effects of total body and tail heating on the peripheral leucocyte count of the rat have been discussed, and the former method is recommended for obtaining blood specimens.[14]

Criteria for characterizing mitochondria by supravital staining with Janus green B have been evaluated, using various substrates, varied concentrations of soluble substrate materials and oxygen tensions, and varied concentrations of the dye. An oxygen-dependent reversible coloration with Janus green B is specific for mitochondria in the cells of both plants and animals. These reactions are dependent on the dehydrogenase and oxidase systems in these structures.[15]

Miscellaneous Techniques.—A microtechnique for hematocrit measurement offers the advantages of small capacity, small over-all size, and a simple method of tube sealing.[16]

A capillary fragility study employing a suction device reports a high incidence of positive results among older people, especially diabetics with hypertension or other vascular disease.[17] The measurement of capillary fragility at its best is still crude.

A method based on the benzidine reaction has been used for the determination of plasma hemoglobin content. Accuracy is limited because of variability of the standard curve with each stock solution of benzidine reagent and the incomplete recovery of hemoglobin from the plasma.[18]

References to other techniques appear throughout the survey.

Immunohematology

The concept that the body is incapable of producing antibody reactive for any of its normal components (horror autotoxicus) remains unaltered, although autoimmunization to abnormal or modified body constituents has been well substantiated. In recent years, considerable interest in autoantibodies has arisen owing to their interference in serologic investigations involving blood grouping and their presence in certain hemolytic anemias. The literature on autoantibody of the last few years has dealt mainly with detection. Recently, however, the research on hemolytic phenomena and autoantibody has dealt more with nature, mechanism, and specificity. This year investigations involving the presence and detection of antiplatelet and antileucocyte immune bodies in hematologic disorders have appeared more frequently in the literature.

Dameshek[19] and others[20] have discussed the possible role of immunologic mechanisms in acquired hemolytic anemia, agranulocytosis, certain platelet malfunctions, acute vascular purpura, periarteritis nodosa, and disseminated lupus erythematosus.

Autoimmunization to Red Blood Cells.—1. Detection of Autoantibody: A biological test, employing the principle of anaphylactic shock, has been reported for the demonstration of erythrocyte-bound antibody in autoimmune hemolytic anemia.[21] Weiner and Gordon described a quantitative test for antibody-globulin coating human erythrocytes. Practical applications include (*a*) testing red cells from cases of autoimmune hemolytic anemia and erythroblastosis fetalis (the degree of coating correlated with the severity of clinical manifestations) and (*b*) determination of the relative number of antigenic points in the discoplasm for agglutinogens of different blood-group systems.[22] Antibody activity of the eluates from red cells in

autoimmune hemolytic anemia has been demonstrated by use of the indirect Coombs, the combined trypsin-indirect Coombs, and the trypsin red cell tests. The two former methods are slightly more sensitive than the latter. Boiling destroys the receptors for these eluted antibodies in human cells.[23]

Abnormal antibodies present in 18 cases of autoimmune hemolytic anemia were of the warm type in 11 patients and of the cold type in 7. Splenectomy was followed by sustained remissions in some of the idiopathic cases of both the warm and cold antibody varieties.[24]

Excellent electron micrographs of intact erythrocytes from a case of autoimmune hemolytic anemia revealed peripheral filamentation across the plasma-corpuscular shrinkage space not present in normal red cells. The filaments were composed of delicate strands of plasma (probably the autoagglutinins demonstrated serologically) adherent to small protruding segments of the surface ultrastructure.[25]

2. Specificity of Autoantibody: Davidsohn and Oyamada emphasize that in testing for autoagglutinins in hemolytic anemia the patient's own cells should be used, as there is significantly greater agglutinability of the patient's erythrocytes, as compared with cells of other persons.[26] Bird makes the same admonishment, suggesting that the tests be carried out in the cold, at room temperature, and at body temperature, not only against O cells but also with the patient's own cells, which would help to exclude cold agglutinins of heterogenetic origin.[27] Weiner and co-workers [28] presented a case of autoimmune hemolytic anemia in which the antibody had the specificity of anti-e. Some doubt is thus cast on the concept that antibodies present in the serum and eluates of patients with this condition are nonspecific, or panagglutinins. It is argued that, for example, an antibody specific for the very common antigen Tj^a (discovered by Levine and co-workers in 1951 [29]) would react with all available test cells, as this antigen is so widely distributed that a cell lacking it might not be available. Therefore, a panagglutinin would be assumed.

Antibody eluted from erythrocytes in autoimmune hemolytic anemia does not react against and cannot be absorbed by red cells of rabbits, guinea pigs, sheep, or monkeys.[23] Dodd, Wright, and others present evidence to the effect that antigenic changes occur in the red cells of patients with hemolytic anemia similar to those produced by trypsin and that these changes are responsible for the characteristic autoimmunization as well as for the specificity and incomplete nature of the antibody found in these cases.[30] Incomplete antibodies in rabbit antiserum to human red blood cells agglutinate trypsinized erythrocytes, presumably because of their capacity to act as divalent molecules in reactions with such cells. Serologic similarities between these systems and those found in human sera in cases of acquired hemolytic anemia suggest a similar explanation for the antibody present.[31] A lytic, agglutinating, and coating factor for trypsinized but not for normal red cells has been found in a normal human serum. This factor differs from the antibodies of acquired hemolytic anemia in that its agglutination of trypsinized erythrocytes is reversible.[32]

Wiener and co-workers ‡ postulate the existence of three major types of autoantibodies. One of these is directed against the nucleus of the ABO substance; the second, against the nucleus of the M-N substance, and the third, against the nucleus

‡ References 22 and 33.

of the Rh-Hr substance. Cold agglutinins appear to be of the type specific for the nucleus of the ABO substance, while warm antibodies (found in most cases of acquired hemolytic anemia) seem to be directed against the nucleus of the Rh-Hr substance.

A case of autoimmune hemolytic anemia with multiple types of antibody, responding to cortisone and corticotropin in different fashions, has been presented.[34] The mechanism of hemolysis in frank hemolytic anemia associated with chronic lymphocytic leukemia is no different from that seen in "idiopathic" autoimmune hemolytic anemia.[35] Methylphenylethyl hydantoin (Mesantoin) therapy resulted in a self-limited acute hemolytic anemia associated with an autoagglutinin and an incomplete panagglutinin. Antibody titers were correlated with severity of disease, and it is felt that the methylphenylethyl hydantoin caused alterations in the patient's red cells, rendering them autoantigenic.[36]

Young and Miller[37] have made a thorough review of the clinical, hemolytic, and serologic differences between hereditary spherocytosis and autoimmune acquired hemolytic disease. Reference to the original article is suggested.

3. Autoantibody and Cold Agglutinins: Formerly, the primary interest in cold hemagglutination was its relation with primary atypical pneumonia. Increasing incidences of hemolytic anemia associated with cold hemagglutinins have been noted in recent years. Human cold hemagglutinin is not identical immunochemically with normal serum gamma globulin.[38] The normal serum panagglutinin active against trypsinized RBC differs from cold agglutinin in the following ways: 1. There is no correlation between cold agglutinin titer and panagglutinin titer; 2. the activity of panagglutinin is not affected by cold, and 3. fractionation of serum shows panagglutinin activity only in Fractions I and III (predominantly alpha and beta globulins), whereas cold agglutinin is found in Fraction II (gamma globulins) exclusively.[39] The incomplete "cold" antibody present in all normal human serum has the specificity anti-H, i. e., reacts with the ABO system quantitatively in the following order (increasing reactivity) A_1B, A_1, B, A_2, O.[40] Complete cold autoagglutinins do not have the specificity anti-H, but anti-H may be a component antibody.[41]

Two cases have been reported in which persistent rather than transient cold agglutinins appears suddenly in high titer and gave rise to prolonged illness characterized by Raynaud's phenomenon, intravascular hemolysis, hemoglobinuria, and gangrene. Cold agglutinins were present in these cases in titers up to 1:327,680 with bovine albumin. Salicylates, corticotropin, and methyl-*bis* (β-chloroethyl) amine hydrochloride (nitrogen mustard) had no effect on reducing the antibody titer.[42] The Raynaud phenomenon was due not only to intravascular agglutination but also to the vasoconstriction which occurs on exposure to cold.[43] A statistically significant relation was found to exist between the occurrence of frostbite and the presence of demonstrable cold agglutinins in the blood, although the titers obtained in this study, for the most part, fell below the range considered normal by other investigators. However, exposure to severe cold and first degree frostbite did not produce cold hemagglutinins in three persons previously negative.[44] The hemolysis associated with the presence of cold agglutinins may be both immunologic and mechanical (high mechanical fragility of RBC) in origin.[45]

4. Autoantibody and Hemolysis: Muratore and co-workers have found that a single injection of a very small dose of anti-guinea-pig-erythrocyte rabbit serum

of low titer is sufficient to cause a rapidly fatal hemolytic anemia and the formation of incomplete autoantibodies in the guinea pig. It is thought that the anemia is not a direct effect of the injected antibody but is caused by the induced autoantibodies.[46]

Autoimmunization and Isoimmunization to Platelets.—A method for testing the potency of antiplatelet rabbit serum, by determining its ability to lyse platelets in the presence of complement in vitro, has been described.[47] Tullis has described a simple in vitro test for the demonstration of platelet antibodies. Criteria for the presence of antibody include (1) quantitative changes in the total platelet count, (2) morphologic alteration, and/or (3) the presence of clumped platelets.[48]

Harrington and co-workers have demonstrated at least eight serologically distinct platelet types. Typing sera consisted of plasma from patients repeatedly transfused for refractory anemia. The incidence of only one type (Type I) was established (18%) by both in vitro agglutination and agglutinin-absorption techniques.[49] Stefanini and Plitman have reported the presence of specific naturally occurring platelet agglutinins in normal humans. In all instances, platelets of the agglutinators were not reciprocally clumped by the plasmas of those whose platelets were agglutinated. Two platelet agglutinogens (I and II) were demonstrated, and, on this basis, four platelet groups have been tentatively proposed: I, 9%; II, 3%; III (I and II), 4%; IV (O), 84% of the white population.[50]

A platelet agglutinin from a case of idiopathic thrombocytopenic purpura was active in agglutinating and interfering with normal platelets at extremely high titers and could be absorbed on and eluted from normal platelets without losing its agglutinating activity.[52] Injection of this patient's serum into normal persons resulted in reactions similar to those reported by Harrington and co-workers [51] in their classic investigations in 1951. Kissmeyer-Nielsen has reported an additional instance of transmissible thrombocytopenic purpura.[53]

Harrington and co-workers, in an outstanding paper, have summarized their evidence that many cases of neonatal and idiopathic thrombocytopenic purpura are results of an immunologic mechanism. Platelet agglutinins have been demonstrated in vitro in plasma of many patients with idiopathic thrombocytopenic purpura. A factor presumably identical to this platelet agglutinin is capable of inducing thrombocytopenic purpura and altering megakaryocytes in normal recipients of this plasma. In cases of immune thrombocytopenic purpura, the spleen probably serves at least two functions, production of some of the platelet agglutinin and removal of some of the sensitized platelets. Neonatal thrombocytopenia is thought to arise in one of two ways: transmission of maternal autoagglutinins across the placenta or the development of isoagglutinins resulting from incompatibilities of the maternal and fetal platelets.[49] Phase-contrast microscopy studies of unfixed unstained materials indicated that the antiplatelet factor of immune thrombocytopenic purpura acts by (1) attacking the platelets being discharged from megakaryocytes, (2) preventing the normal formation of platelets, and (3) preventing the normal development of megakaryocytes.[54]

The recent advances in idiopathic thrombocytopenic purpura have been reviewed and a comparative classification with hemolytic anemia has been proposed by Stefanini and Dameshek [55] (see Table 3, under Platelets).

The mechanism of formation of platelet antibodies (agglutinins, lysins) destructive to the patient's own platelets is unknown at present. It is conceivable, however, that bacteria, viruses, and certain other substances might modify the structure of

platelets and give them autoantigenic properties. Motulsky has demonstrated virus platelet agglutination inhibition similar in all respects to the well-known phenomenon of virus hemagglutination and virus-hemagglutination inhibition.[56] In view of demonstrations that virus-modified erythrocytes may act as antigens in the production of autoimmune acquired hemolytic anemia, the elucidation of virus-platelet agglutination suggests that virus-modified platelets may play a similar role in the production of platelet antibodies in certain cases of "immune" thrombocytopenic purpura. A case of thrombocytopenic purpura with gross hemorrhage subsequent to measles has been described and likened to the "allergic" purpura of allylisopropylacetylcarbamide (Sedormid).[57]

The mechanism of thrombocytopenic purpuras resulting from certain drugs is hypothetical. There is, however, evidence suggesting a hypersensitive mechanism, with the antigen-antibody reaction in some way inducing the thrombocytopenia. Among the drugs incriminated in this type of thrombopenic purpura are allylisopropylacetylcarbamide,[58] quinidine,[59] digitoxin,[60] and sulfamethazine (sulfamezathine) and quinine.[61]

Autoimmunization to Leucocytes.—Specific human and guinea pig leucocyte lysis has been produced by the addition of homologous antigen to fresh sera of rabbits immunized with crystalline hen ovalbumin or with bovine gamma globulin. The cells affected by specific lysis are polymorphonuclear neutrophiles.[62] Heteroimmune antibody has been produced in rabbits against human granulocytic leukemia leucocytes. The antibody produces in vitro agglutination, lysis, and phagocytosis of normal human granulocytes by monocytes and polymorphonuclear neutrophiles. Many of the cells produced on incubation with antileucocytic serum appear identical with the Hargraves "L. E. cell." § This suggests that the L. E. factor may be related to an autoimmune leucocyte antibody.

Hansen has reported a case of severe agranulocytosis accompanied by a massive elevation of gamma globulin. Treatment with corticotropin resulted in depression of gamma globulin well correlated with clinical and hematologic correction. It is proposed that the disease may be the leucocytic "variant" of immunologic hemolytic anemia.[65] Dausset and co-workers ‖ have found leucoagglutinating substances in the sera of patients with pancytopenia and leucopenia, and Kissmeyer-Nielsen [53] has demonstrated the presence of a transmissible leucopenic factor in the serum of a patient with leucopenia. Moeschlin concludes, from a detailed clinical and experimental study, that certain forms of agranulocytoses and leucopenias have immunologic mechanisms.[68]

Thus, the demonstration of leucoagglutinins completes the triad of specific autoimmune substances for the three elements of human blood: antierythrocyte, antithrombocyte, and antileucocyte agglutinins. Further studies are needed to characterize and elucidate the immunologic nature.

General Observations on Blood Types.—Wiener [69] describes a universal method for designating blood factors, agglutinogens, genes, phenotypes, and genotypes, using the Duffy system as a model.

Differences in pH of the diluents employed may contribute to variations in the results of anti-Rh tests.[70]

§ References 63 and 64.

‖ References 66 and 67.

Titers of alpha$_2$ isoagglutinins detected at 37 C. and those acting at 6 C. in the serums of monkeys and rabbits injected with polysaccharides from animal parasites were reduced to negative for 24 hours and remained lower than normal for 96 hours.[71] Bird has reported the demonstration of dual-receptor antibody structure (anti-A—anti-B linkage) in human Group O sera and has suggested that similar linkage (multireceptor antibody structure) often occurs in the case of autoagglutinins and renders the various component antibodies inseparable by absorption.[72]

Methods have been reported for (1) the determination of blood groups in emergency situations,[73] (2) recording blood groups rapidly and accurately,[74] and (3) detecting the D^u factor.[75] The necessity for adding albumin to serum in crossmatching tests is emphasized, although this method cannot be relied upon for the detection of incompatibility due to the anti-K, anti-Fy^a, or anti-Jk^a antibodies or in cases in which the D^u factor is involved.[76] The possibility of obtaining false blood group results on cord blood of babies is reported to be small.[77] Relations are described between the ABO blood groups and (1) age-groups,[78] (2) fertility,¶ and (3) certain malignant [82] and nonmalignant [83] diseases. The use of blood-grouping tests for exclusion of paternity is reviewed.[84] Occurrence of substances similar to several of the classic human blood group antigens has been noted in the chimpanzee,# but they do not occur in the cat [87] or the monkey.[86]

Knowledge of the blood group systems has been applied to problems involving anthropology [88] and ethnic groups [89] in studies on Australian Aborigines [90]; Blackfoot Indians of Canada [91]; Central,[92] Southern,[93] and Western [94] Africans; Eskimos [95]; Gilbertese,[96] and Nigerians.[97]

Genetics of Blood Types.—The first known case of a double set of human blood groups (O and A_1) has been reported in a woman. One of the sets of blood groups was attributed to the consequences of vascular anastomosis with her male twin in utero.[98] An allele of the MN blood group system, M^c,[99] and two genes closely linked to the MNSs system [100] have been demonstrated. The properties of the M^c gene are almost completely analogous with those of the c^v gene in the Rh system. Studies on linkage between the genes for elliptocytosis and the genes for various blood types gave negative results in two * of three cases.[103] Homozygous D^u (D^u/D^u) has been demonstrated in a family with uncommon Rh genotypes.[104]

Rare Blood Groups and Factors.—Family antigens or "private" blood group classifications (characterized by the positive or negative findings being limited to members of a particular family) are discussed and criticized.[105] Two new "private" factors, Be^a [106] and Wr^a [107] are reported.

The significance of the newer blood factors encountered in blood transfusions has been examined,† and Rosenfield and co-workers [110] describe a crossmatching technique capable of detecting "every" human isoantibody thus far described. Rosenfield and Vogel review the newer knowledge of human blood factors and present some of the applications of the various blood group systems in clinical medicine.[111]

Wiener presents evidence for a third isoagglutinin, anti-C, and its corresponding factor, C, in the ABO system. The incidence of Group C is about 1 in 60,000.[112] A new Rh antibody, anti-f, and the antigen it defines has been reported [113] and dis-

¶ References 79-81.
# References 85 and 86.
* References 101 and 102.
† References 108-110.

cussed.[114] A second example of the Rh agglutinogen c^v has been reported, and it is suggested that the agglutinogen f (above) is a position effect of the genes c and e.[115] Additional examples of the anti-Jk^a hemagglutinin ‡ have been reported; quantitative aspects of the Duffy ^{a} (Fy^a) antigen have been studied,[118] and isosensitization to the Kell factor has been investigated.[119] Natural (erythroblastosis) and artificial (transfusion) isoimmunization phenomena have resulted in the discovery of a new blood-group antibody, anti-Jk^b,[120] and a new blood factor, U.[121]

The main blood group systems, their known antigens, and their relation to hemolytic transfusion reactions are summarized in Table 1.

Hemolytic Phenomena Due to Transfusion Isoimmunization.—Unnecessary transfusions with severe subsequent reactions continue to be reported and pleas are made for adequate cross matching procedures before transfusions.§ Cross matching techniques capable of detecting the most significant human isoantibodies have been described.‖ The legal responsibility in blood transfusion reactions has been discussed.[126]

TABLE 1.—*The Main Blood Group Systems: Their Antigens and Their Relation to Transfusion Reactions**

System	Antigens	Association with Transfusion Reactions
ABO	O, A_1, A_2, B, C	Common
MNS	M, N, S-s	Rare
Rh	D-D^u-d, C-C^w-e^v-e, E-e, f	Common
P	P	None reported
Kell	K, k	Rare
Lutheran	Lu^a	Doubtful
Lewis	Le^a, Le^b	Very rare
Duffy	Fy^a, Fy^b	Rare
Kidd	Jk^a, Jk^b	Yes

* Modified with additions from Sacks,[108] *Annals of Internal Medicine*, The American College of Physicians.

Cross transfusions were done in patients with toxemias of pregnancy, acute renal shutdown, and uterine hemorrhages of puberty. Twenty per cent of such transfusions attempted resulted in undesirable side-reaction.[127]

Hemolytic reactions resulting from incompatible transfusions have been found to be caused by (1) anti-A,[128] (2) anti-Jk^a,[117] (3) anti-Jk^b,[120] (4) anti-K,[119] (5) anti-c,[129] (6) anti-U (a new factor),[121] and (7) negligence.[130] A hemolytic reaction due to incompatible transfusion was successfully treated by massive replacement transfusion with compatible blood.[131]

Levine [109] and Sacks [108] have reviewed the significance of the new red cell antigens in transfusion therapy. The major blood group systems known to be involved in hemolytic transfusion reactions are presented (Table 1).

Erythroblastosis.—A number of reviews of erythroblastosis cover the incidence of antibody in first pregnancies,[132] the frequency factors involved, treatment, and complications.¶

‡ References 116 and 117.

§ References 122-124.

‖ References 110 and 125.

¶ References 133 and 134.

In rabbits, antibodies to erythrocyte factors, whether produced by the injection of red cells or by pregnancy, cross the placenta readily from the mother to fetus and are found at birth associated with the red cells and in the serum of the fetuses. The rabbit placenta appears to be equally permeable to agglutinating and coating antibodies.# Observations have been made in pigs.[137]

Two methods for the detection of weak Rh antibodies are (1) the use of papain-treated Group O Rh+ cells [138] and (2) sera containing Rh antibodies undetectable by the standard procedures, causing an increase in titer when used as diluents in the titration of known anti-Rh sera (this effect is negated by previous absorption with O Rh+ cells but not with O Rh— cells).[139]

Several cases of deficient prothrombin and fibrinogen in fatal erythroblastosis fetalis [140] and three cases of hypofibrinogenemia occurring in Rh-isosensitized multigravidae carrying dead fetuses [141] have been reported.

Isoimmunization of pregnant mothers to ABO has resulted in many cases of erythroblastosis.* Dunn [143] emphasizes the fact that maternal isoimmunization occurs in a large number of normal pregnancies without affecting the fetus. The osmotic fragilities of erythrocytes from babies with erythroblastosis due to anti-A are higher than when anti-Rh is involved. The distribution of the ABO blood groups among sensitized Rh— mothers has been compared with the distribution among nonsensitized Rh— mothers. The frequency of group A_1 was significantly higher than the frequencies of Groups O, B, and A_2 in the series of sensitized mothers.[148] Sensitization to the c (hr′) factor, the Jk^a factor,[117] and new "private" factor, Be^a,[106] occurred.

Cortisone was administered during the last six months of pregnancy to a woman with a markedly elevated anti-Rh titer whose second child had died of hemolytic disease of the newborn. Although the cortisone markedly reduced the anti-Rh titer of the patient, her third child died of erythroblastosis fetalis two hours after birth, with the characteristic findings of extramedullary hemopoiesis in the spleen and liver.[149] Routine premature induction of labor increases mortality.[150] Exchange transfusion is still the most effective therapy for erythroblastosis.[151] A method and the equipment employed therein have been described.[152]

Bone Marrow

Many studies on the basic nature of bone marrow in both normal and pathologic blood states have been reported. The dye-binding capacity of blood and bone marrow cells of man has been studied, and the effect of various chemical and physical factors influencing the uptake of dye has been noted. The pH of the staining solution is the most important factor influencing dye uptake.[153] No important sudanophilic staining differences have been demonstrated between normal marrow preparations and those obtained from persons with a variety of blood disorders.[154] From studies on the mean duration of mitosis in erythroblasts and leucoblasts [155] and determinations of the number of active hemopoietic cells in normal subjects, Journoud and co-workers conclude that classic theories of granulopoiesis and erythropoiesis are incapable of explaining the production of mature granulocytes and erythrocytes.[156] Four blood coagulation factors in bone marrow extract have been demonstrated by paper chromatography.[157] The Kety radioisotope clearance tech-

References 135 and 136.

* References 142-147.

nique with radioactive iodine (I^{131}) demonstrates a selective increase in blood flow in the bone marrow in acute leukemia and in some cases of chronic lymphatic leukemia and multiple myeloma.[158] The chemical composition of rabbit bone marrow is related to histologic findings in starvation [159] and experimentally produced hemolytic anemia.[160] Bone marrow studies of house mice with genetic macrocytic anemia show a defect in blood formation.[161]

Boll has studied the in vitro effect of the mitotic toxins, colchicine and xanthopterin, on bone marrow tissue cultures and has found that colchicine acts primarily on erythroid cells and xanthopterin acts more on the myeloid elements.[162] A new type of chemically produced abnormality of mitosis appeared after an injection of hydroquinone in the intestinal germinative cells of the mouse and was observed in the bone marrow of the golden hamster in the first six hours after an intraperitoneal injection of the drug.[163] Single subcutaneous injections of cortisone in hypophysectomized and normal rats resulted in (1) a decrease in the percentage of immature granulocytes within the marrow, (2) a depression in bone marrow oxygen consumption, and (3) an increase in eosinophilic elements in the hypophysectomized rats and an increase in lymphocytes in the normal rats. The relation of these changes to cortisone-induced peripheral eosinopenia, lymphopenia, and polymorphonuclear leucocytosis is not known.[164] Failure of bone marrow to respond to the stimulus provided by bacterial pyrogen may reveal deficiencies in marrow function not evident by routine white blood cell count. This may be useful in evaluating bone marrow status after use of nitrogen mustard.[165] Gamma irradiation produces a marked decrease in the concentration of ribonucleic deoxyribonucleic acids in the cellular elements of exposed bone marrow of rabbits.[166]

Aspiration and Preparation Techniques.—Berman has presented an excellent review on methods of aspiration, biopsy, and examination. Bone marrow biopsy is indicated whenever a disease affecting the hemopoietic system is present or suspected and diagnostic information is not available.[167]

The tibia has been designed as the site of choice in children under 2 years. The sternum is a preferable site for cooperative patients of all ages, and the ileum is readily accessible in the apprehensive patient.[168] A simple method suitable for repeated marrow aspirations in the unanaesthetized monkey has been described.[169] An improved hematoxylin and eosin stain for sections of marrow units [170] and an improved method for preparing marrow particles for sectioning [171] have been mentioned. Careful examination of a good marrow aspirate often provides diagnosis or a strong lead in difficult diagnostic problems.[172] Grossly black aspirates or black granules in the cytoplasm of tumor cells were found in four of eight patients with metastatic malignant melanoma.[173] Bone marrow plasmacytosis may aid in diagnosis of multiple myeloma, although other diseases, such as rheumatoid arthritis, hepatic cirrhosis, Hodgkin's disease, and granulomatous and collagen diseases, may be associated with plasma cell increases.[174] Examinations of the marrow may be of diagnostic value when x-rays show miliary pulmonary opacities or diffusely distributed pulmonary lesions.[175] Of a series of 24 patients with such roentgenographic evidence, 9 showed a pathologic lesion in the marrow, and, of these, 3 had miliary tuberculosis; 2, sarcoidosis; 2, histoplasmosis; 1, amyloidosis, and the condition of one remained undiagnosed. Routine marrow culture for Histoplasma capsulatum is of value in children but not adults.[176]

Toxic Depressants.—Osgood has made a superb review of the drug-induced hypoplasias and summarizes that 1. there is a time lag between first exposure and onset of symptoms; 2. repeated or prolonged exposures result in increased incidence; 3. severity is rarely related to dosage, and 4. many persons with hypoplastic states have hypersensitivities. The majority of toxic depressants belong to the following groups: anticonvulsants, antihistamines, antimicrobial agents, heavy metals, sedatives, and spasmolytics.[177]

Bone marrow depression following chloramphenicol (Chloromycetin) therapy may involve erythropoiesis, myelopoiesis, and/or thrombocytopoiesis.[178] This is still the drug of choice in typhoid fever.[179] A temporary depression may follow short-term therapy.[180] Irreversible changes with fatal aplastic anemia may result from small, intermittent doses.† Aplastic anemia has also followed administration of quinacrine (Atabrine),[183] intermittent streptomycin and daily *p*-aminosalicylic acid,[184] and trinitrotoluene exposure eight years previously.[185] Other hypoplastic anemias are associated with arsenobenzols, methylphenylethylhydantoin (Mesantoin), gold preparations, sulfonamides, and trimethadione.[177] Bone marrow studies of selenium-treated ducks demonstrate a hypoplasia of erythroid elements. This depressant activity is counteracted by cysteine, which indicates that an enzyme system dependent on the sulfhydryl group is inhibited.[186]

Severe pancytopenia has been observed following therapy with (1) radioactive iodine (I^{131}),[187] (2) methylphenylethylhydantoin and hydralazine (Apresoline),[188] (3) 3-methyl-5-phenylhydantoin (Nuvarone),[189] and (4) two other hydantomates, methylphenylethylhydantoin and diphenylhydantoin (Dilantin).[190] Hypoplasia of myeloid elements has been reported following the use of (1) methimazole (Tapazole) and propylthiouracil[191]; (2) nitrophenols, thiosemicarbazones, antihistamines, phenothiazines, and procaine amide hydrochloride,[177] and (3) phenylbutazone (Butazolidin), used for symptomatic relief of arthritis.‡ Bone marrow studies obtained at autopsy from six persons who had received more than 50 mc. of radioactive colloidal gold (Au^{198}) demonstrated hypoplasia of myeloid elements.[198] Hahn, however, reported no significant bone marrow changes in several hundred patients treated with colloidal gold.[199]

p-Aminosalicylic acid (PAS), streptomycin,[200] and mercurial diuretics[177] have been shown to produce thrombocytopenia.

Other Bone Marrow Failures.—The entire picture of bone marrow failure has been a discouraging one. The lack of understanding of the fundamental processes involved has resulted in confused thinking and confused terminology. The all too frequent fatalistic attitude of both the physician and patient is understandable.

Smith has competently reviewed the hypoplastic and aplastic anemias of infancy and childhood and classifies them into three groups, recognizing that there is overlapping.[201] These are (1) pure red-cell anemia, (2) hypoplastic anemia, and (3) aplastic anemia. Pure red-cell anemia is chronic aregenerative anemia in which the failure of the bone marrow is confined to erythropoiesis without simultaneous depression of the granulocytes, platelets, or their precursors. Hypoplastic anemia is associated with impairment of red cell formation with varying degrees of involvement of granulocytes and platelets. In aplastic anemia, the three marrow elements are depressed, resulting in pancytopenia peripherally.

† References 178, 181, and 182.

‡ References 192-197.

One of the outstanding contributions has been made by Loeb, Moore, and Dubach, on the physiologic evaluation and management of chronic bone marrow failure.[202] They evaluated erythroid production and erythroid destruction in 10 patients. Erythropoiesis was determined by the red blood cell counts, hemoglobin levels, reticulocyte counts, efficiency of radioactive iron utilization, and examination of the bone marrow. Erythrocyte destruction was studied by measurement of hemoglobin catabolism as reflected by the fecal urobilinogen excretion and a calculated hemolytic index and by determination of the survival time of transfused erythrocytes. They demonstrated that the anemia of chronic bone marrow failure is not only due to a decreased production of erythrocytes but may be frequently associated with a hemolytic component. Erythropoiesis in some patients may be stimulated by the administration of corticotropin or cortisone. Those patients responding to hormonal therapy are good prospects for splenectomy. When a hemolytic component is present, splenectomy tends to correct the abnormal erythrocyte destruction.

One patient with chronic erythrocytic hypoplasia showed improvement after oral cobalt chloride therapy.[203] Holly reports eight cases of hypoplastic anemia occurring during pregnancy and hypothesizes that some substance is elaborated during pregnancy producing a depressing or toxic effect on the marrow of the rare sensitive person.[204]

Splenectomy has generally been regarded as contraindicated in myeloid metaplasia of the spleen. Green and co-workers have reviewed the documented material as well as five cases in their own experience in which splenectomy had been done. They conclude that splenectomy does not appreciably affect the general status in most instances, with neither improvement nor "failure of the blood-forming system." However, there is a rare instance (associated with thrombocytopenia or hemolytic anemia) that may be definitely benefited by splenectomy.[205]

Development of thrombocytopenic purpura following resection for carcinoma of the bronchus has been observed as the first sign of metastases.[206]

Irradiation

Immediate Effects.—Bilobed or double nucleated lymphocytes resembling those found in animals and humans exposed to neutron radiation have been found in the peripheral blood of rabbits after x-irradiation.[207] Nuclear and motility changes have been observed in human lymphocytes following in vitro gamma irradiation.[208] An apparatus is described with which proton microbeams can be used to bombard small fractions of a single cell in vitro. The effects on such cells are then observed by time-lapse microcinematography.[209]

Dependence of irradiation dose on body size in initial acute postirradiation symptoms may be influenced by a diffusible metabolite.[210] An "artificial kidney" was found ineffective in reducing mortality of early postirradiated dogs.[211] This observation minimizes the possibility of a dialyzable toxin in the lethal action of x-irradiation.

Reduction of carboxypeptidase inhibitor in blood cells may serve as a basis for determining whether a person has received a lethal dose of ionizing radiation within 24 hours of exposure.[212] A large absolute and relative eosinophilia has been observed in monkeys during the fifth and seventh weeks following an LD 50/30 total body irradiation.[213] The remainder of the blood picture also closely parallels that observed

in lower animals. Total body lethal gamma radiation in burros gives the following pathologic changes: severe thrombocytopenia, retardation of whole blood clotting time, lessening of clot retraction, and diminution of prothrombin utilization. No demonstrable circulating anticoagulant is found.[214]

The effects of ionizing radiation on the reticuloendothelial system remain controversial. Gabrieli and Auskaps have found that reticuloendothelial capacity is diminished by x-irradiation, and they suggest that the reticuloendothelial system be considered a highly radiosensitive tissue [215]; this is contrary to the generally held opinion. Following x-irradiation in mice, no significant effect on the splenic uptake of thorium dioxide (Thorotrast) was noted.[216] Injection of radioactive-iodine (I^{131})-labeled antigen in x-irradiated rats with and without spleen shielding results in no difference in rate of phagocytosis, tissue localization, or rate of disappearance of antigen from splenic tissue.[217] Striking erythrophagocytosis has been found in lymph nodes of dogs one hour after total body irradiation.[218]

Long-Range Effects.—A summary of clinical observations for the first six months immediately following atom-bomb-induced radiation sickness at Hiroshima has been presented.[219] Hematologic values of Hiroshima survivors recorded in the 33 to 44 months following exposure were not significantly different from normal.[220] Another survey of midlethal doses of total body ionizing radiation has been made by De Coursey.[221]

Protection and Therapy.—Excellent reviews of the mechanisms involved and the substances used for protection against the effects of ionizing radiation have been made by Brues and Patt,[222] Rugh,[223] and Patt.[224] Factors shown to have no demonstrable protective value and those detrimental to survival following irradiation are itemized.[223]

Postirradiation protection activity of mouse spleen hemogenates is associated with the cell nucleus fraction.[225] Similarly, the beneficial effect of bone marrow emulsion in irradiated rabbits does not depend on growth of the injected cells but rather on a noncellular fraction.[226] Protective factors in spleen and bone marrow seem to be closely related in nature or even identical—perhaps nucleoproteins.[225] Nonirradiated bone marrow and spleen of C57 black mice contain an active principle that accelerates the regeneration of both radiation-involuted thymus and lymph nodes and inhibits the development of thymic lymphoid tumors.§ The effectiveness of spleen homogenate in increasing survival of irradiated mice is thought to be due to increased resistance to infection.‖

Dogs receiving whole blood transfusions after sublethal doses of x-irradiation showed no deleterious effects; this is contrary to previous findings. It is possible that an incompatible transfusion, not fatal to the normal dog, may effect an injury lethal to some irradiated animals.[231]

A single transfusion of concentrated homologous blood platelets eliminates the large-scale diversion of erythrocytes into the lymph of rats and dogs during the hemorrhagic phase of irradiation injury.[232] Soybean phosphatide is of some therapeutic value as a substitute for the platelet factor in dogs made thrombocytopenic by total body x-irradiation.[233] The protective value of parabiosis in postirradiated rats has been confirmed.[234]

§ References 227 and 228.

‖ References 229 and 230.

Injection of 2-amino-ethanethiol (β-mercaptoethylamine; mercaptamine; cysteamine) prior to total body irradiation in mice gives protection from radiation syndrome.[235] The white cell count and body weight of 2-amino-ethanethiol-protected mice drops during the two or three days following irradiation and then increases. Injection of 2-amino-ethanethiol after irradiation is not only ineffective in preventing death[236] but even increases mortality.[235] Intravenous injections of 2-amino-ethanethiol (200 mg.) often eliminate symptoms of irradiation sickness found in cancer patients treated by ionizing radiation.[236]

Mortality in irradiated laboratory animals is not reduced by substances such as citrovorum factor,[237] polyvinylpyrrolidone (in combination with Macrose, a synthetic plasma expander, and dextrose),[238] an orally administered nonprotein nonanaphylactogenic bacterial pyrogen (Piromen, Baxter),[239] and somatotrophic hormone.[240] Intraperitoneal injections of Piromen given before total x-irradiation increase the mean survival time, although postirradiation treatment decreases survival time.[241]

A cobalt-containing diet,[242] essential fatty acids, or defatted liver powder[243] increase the resistance of mice to irradiation.[242]

Numerous studies on the susceptibility of irradiated animals to infection and the therapeutic effects of antibiotic treatment have been made.¶ Therapeutic effects of antibiotic treatment in swine receiving x-irradiation and thermal burns (simulating the atomic bomb) have been evaluated.[249]

Corticotropin (ACTH) and Cortisone

Techniques.—A method for the direct counting of circulating eosinophiles in corticotropin (ACTH) and epinephrine (Adrenalin) tests has been evaluated and found wanting.[250] A method for the assay of corticotropin in either intact or hypophysectomized mice has been proposed.[251] Evaluation of changes in the eosinophile levels in studies of adrenocortical function and stress in humans emphasize the importance of subjective factors.[252] Repeated eosinophile counts are of no value in determining the effective dose of adrenocortical steroids.[253]

Experimental Studies.—The relation of epinephrine to adrenocortical steroids in the production of eosinopenia has been discussed.# Speirs believes that epinephrine acts on eosinophiles through a release of hormone from adrenal cortex tissue.[258] Epinephrine appears to be more effective in inducing eosinopenia than arterenol (Nor-epinephrine).[259] Decline in eosinophiles is reported to be dependent on the presence of the hypophysis.[260] N-phenoxyisopropyl-N-benzyl-β-chloroethylamine hydrochloride (Dibenzyline) does not inhibit the prolonged leucocyte response to epinephrine.[261] Pyridoxine-deficient rats display normal eosinopenic responses to epinephrine, corticotropin, and hypoxia.[262] Adrenal hormones act only indirectly in the destruction of eosinophiles.[263] The bone marrow probably is not responsible for the immediate eosinopenic response to corticotropin.[264] Hormonally induced eosinopenia of rat peritoneal fluid has been studied.[265] "Amparon," a casein hydrolysate, produces hematological changes similar to those produced by cortisone in hypophysectomized rats.[266]

¶ References 230, 240, and 244-248.

References 254-257.

Eosinopenia has been reported to follow prolonged exercise in man,[267] ingestion of casein or tyrosine in man,[268] fasting in dogs,[269] venesections repeated at intervals of several days in mice,[270] and administration of morphine in humans.[271] Eosinopenia also has been observed during the last third of pregnancy in mice.[272] Diurnal eosinophile fluctuation is maintained in mice exposed to continuous cold.[273] Eosinopenia from epinephrine can be abolished in the cat by hypothalamic lesions.[274] The functional state of the reticuloendothelial system probably has no great influence on the eosinopenic response observed in stress.[275] Blood sugar and eosinophile response in rats to 30% carbon dioxide in air and in oxygen has been studied.[276] Two types of lymphocytes have been observed in the normal mouse—normal lymphocytes and stress lymphocytes (large cells with increased amounts of poorly basophilic cytoplasm)[277]; the lymphocytic response varies inversely with the amount

TABLE 2.—*Therapeutic Evaluation of Corticotropin (ACTH) and Cortisone in Blood Disorders**

Definite Therapeutic Value	Limited or Uncertain Therapeutic Value	No Therapeutic Value
Acute lymphocytic leukemia Acute myelogenous leukemia Acute stem cell leukemia	Hodgkin's disease Lymphosarcoma Leukemia cutis Mycosis fungoides Multiple myeloma	Acute monocytic leukemia Chronic monocytic leukemia Chronic myelogenous leukemia Chronic lymphocytic leukemia Plasma cell leukemia
Idiopathic acquired hemolytic anemia (immunohemolytic or hypersplenic)	Chronic aplastic anemia (rarely)	Acquired hemolytic anemia due to direct destruction of erythrocytes by chemicals, etc.
Secondary acquired hemolytic anemia in conditions responding to ACTH or cortisone	Simple chronic anemias due to conditions responding to ACTH or cortisone Sickle cell anemia	Congenital hemolytic anemia Acute aplastic anemia Chronic aplastic anemia Simple chronic anemia Myelophthisic anemias Pernicious anemia Macrocytic anemias of other types Anemias due to iron deficiency and blood loss
Idiopathic thrombocytopenic purpura Nonthrombocytopenic purpura (anaphylactoid)	Secondary thrombocytopenic purpuras due to conditions responding to ACTH and cortisone	Secondary thrombocytopenic purpuras due to conditions which do not respond to ACTH and cortisone ? Thrombotic thrombocytopenic purpura

* From Manning,[295] *GP*, The American Academy of General Practice.

of adrenocortical hormone in the blood.[278] Trowell claims that cortisone destroys rat lymphocytes in vitro.[279] The lymphopenic response is impaired following repeated injections of epinephrine into pyridoxine-deficient rats.[280] New cell formation and cellularity in the bone marrow, spleen, and thymus decline progressively in rats treated for several days with corticotropin.[281] Lymphatic tissue protein may be made available for metabolic use by adrenocortical mediation.[282]

Hypophysectomy in rats results in decreased oxygen consumption by bone marrow.[164] Intravenous phenylhydrazine causes greater red cell destruction in adrenalectomized rats than in controls.[283] Studies on hemoglobin excretion in dogs did not help to elucidate the effects of corticotropin therapy in the nephrotic syndrome.[284] Swingle and co-workers suggest that cortisone helps to maintain a normal circulation by its action on the vascular periphery.[285] Adrenocortical steroids in human peripheral and adrenal venous blood have been studied by means of fluorescent materials.[286]

Clinical Observations.—Wight and co-workers state that eosinophile counts in burned patients can be used prognostically. Failure of eosinophiles to reappear (following the immediate eosinopenia of burns) by the third postburn day may indicate a grave prognosis.[287] Criteria for evaluating the adequacy of adrenocortical function by eosinophile counts in major surgery patients postoperatively have been outlined by Schoen and co-workers.[288] Another sign, the lack of response of circulating eosinophiles to epinephrine, has been added to the hypothalamopituitary syndrome of oligospermia and impaired dark-adaptation.[289] Endogenous eosinopenia (decrease in eosinophiles during the morning hours) found in mentally deficient patients was thought to indicate the presence of functioning adrenal cortices.[290] Eosinophile levels of blood of psychopathic persons are greater than those of mentally normal persons.[291] Eosinophilia results from full-thickness homografting in man.[292] The peripheral blood levels of 17-hydroxycorticosteroids in health and disease and the response of 17-hydroxycorticosteroid blood levels to injections of corticotropin, cortisone, and epinephrine have been studied in children.[293]

Therapy.—Ragan presents a valuable general review of the clinical use of cortisone and corticotropin.[294] Several critical discussions of their therapeutic value in various blood disorders have appeared.* A therapeutic evaluation of corticotropin and cortisone is presented (Table 2). Specific uses of these hormones are found in the appropriate sections of this survey.

Hematologic Problems in Pregnancy

Many hematologic diseases occur in pregnancy. Discussion of these is scattered throughout the survey. Regrouping of these references here emphasizes the extensiveness of this relation. Pregnancy and the following hematologic states have been considered: erythroblastosis fetalis,† erythrocyte sedimentation rate,[298] hypoplastic anemias,[204] total red cell mass,[299] iron deficiency anemia and iron metabolism,‡ hemolytic anemia,§ sickle cell anemia,‖ macrocytic anemia,¶ thrombocytopenic purpura,# Gaucher's disease,[315] leukemia,* Hodgkin's disease,[321] and secondary polycythemia.[322] A mild to severe anemia in pregnant rats does not cause any striking effects in the fetus during gestation.[323]

(To be continued)

* References 295-297.
† References 106, 117, 132-134, and 140-151.
‡ References 300-303.
§ References 304-306.
‖ References 307-310.
¶ References 311 and 312.
# References 313 and 314.
* References 316-320.

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(To be continued)

Case Reports

BACTERIAL ENDOCARDITIS

Unusual Case with Blood Cultures Positive for Brucella Abortus and Viridans Streptococcus

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THE CLINICAL, bacteriologic, immunologic, and pathologic criteria necessary for establishing the diagnosis of bacterial endocarditis are difficult to fulfill. Fortunately, since the advent of antibiotics the mortality due to bacterial endocarditis has diminished markedly, so that the majority of patients now recover, whereas previously recovery was a rare event. At the autopsy table it was possible to demonstrate the characteristic valvular vegetations and to culture the causative bacteria from the vegetations. At the present time more and more reliance must be placed on the clinical, bacteriologic, and immunologic findings in an effort to establish the correct diagnosis and the etiologic bacteria, because most patients properly treated do not reach the pathologist. Correct bacteriologic diagnosis is a necessity, because the choice of antibiotic to be employed in treatment depends on the particular bacteria responsible for the infection.

There are very few reported cases of bacterial endocarditis due to Brucella. Smith and Curtis [1] in 1939 reviewed nine previous cases, including four cases of undulant fever in which the heart was affected. These latter four cases were reported by Hughes in 1897 [2]. Smith and Curtis reported another case. Spink and Nelson [3] in 1939 were able to find only two cases in the literature substantiated by anatomical and bacteriological evidence at necropsy. Spink and Nelson [3]; Spink, Tritud, and Kabler,[4] and Wechsler and Gustafson [16] each reported a case of bacterial endocarditis due to Brucella, and Spink and associates in a series of 84 bacteriologically proved cases had 4 with subacute bacterial endocarditis.[18] Jones [5] searched the literature of the 13-year period 1936-1948, inclusive, for cases of subacute bacterial endocarditis of nonstreptococcal etiology, and she lists 18 references of endocarditis due to Brucella. In 15 of the references, case histories were detailed, but study of these references reveals that few were proved cases.

The natural course of brucellosis makes differentiation from subacute bacterial endocarditis difficult; particularly is this true in patients with congenital or rheumatic heart disease. Both brucellosis and subacute bacterial endocarditis may be characterized by gradual onset of malaise, fever, anorexia, chills, arthralgia, arthritis, and splenomegaly. These findings in a patient with cardiac murmurs consistent with

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valvular lesions and a blood culture positive for Brucella would leave one confused as to whether the patient had subacute bacterial endocarditis due to Brucella or brucellosis without bacterial endocarditis, although experience would favor that latter.

Bacterial endocarditis with mixed infection due to two or more different bacteria is uncommon. Orgain and Poston [6] in 1942 reported 27 cases of bacterial endocarditis, 2 due to Brucella suis and 6 in which there was a mixed infection, but in none of these cases of mixed infection was Brucella one of the organisms cultured from the blood stream.

The following case is reported because of the unusual occurrence of bacterial endocarditis in a patient in whom two organisms, viridans Streptococcus and Brucella abortus, were cultured from the blood stream. The patient is of further interest because recovery apparently occurred following the administration of a combination of chemotherapeutic and antibiotic agents. Penicillin was not employed because the patient was sensitive to it.

REPORT OF A CASE

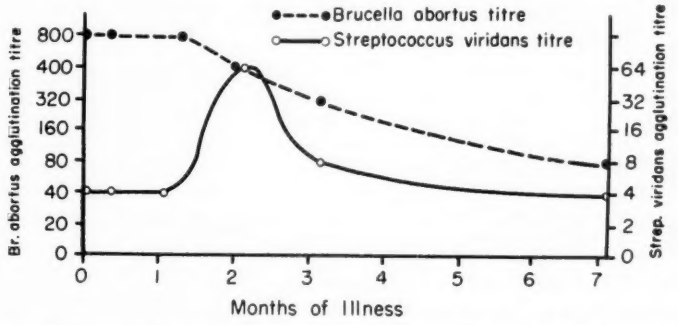
E. S., a 25-year-old married white male student veteran, was admitted to the University Infirmary on Jan. 14, 1951, with the chief complaint of fever and headache. The history of the present illness began early in December, 1950, when he noted excessive fatigue after shoveling snow. Fatigue continued as the principal symptom for a week. Just before Christmas the patient's father became ill and the patient went home to help on the farm. Just after Christmas a dry, nonproductive cough began, and, for the first time, on Dec. 28 he felt feverish. He reported to the Veterans Administration physician and was given one of the sulfonamides for six days (exact drug not known). Immediately after taking the sulfonamide he began to perspire and his temperature returned to normal. The next day, after he stopped taking sulfonamides his temperature rose to 102 F., but it again returned to normal when he resumed taking the sulfonamides. He continued to complain of fatigue and headache and now had severe malaise. On Jan. 5 and 6, 1951, he received 400,000 units of penicillin intramuscularly daily (preparation unknown) from his private physician. On Jan. 6, in the morning, generalized urticaria with hives occurred, followed by exfoliation of the hands and tongue. His temperature ranged between normal and 103 F. but usually returned to normal after he took sulfonamides. On Jan. 14 his private physician suspected that the patient had undulant fever and referred him to the infirmary, to which he was admitted. Further questioning at this time revealed that the patient was in the habit of frequently visiting his father-in-law's dairy farm, in southern Wisconsin, on weekends, where he helped milk cows. He did not drink raw milk but did use it in his cereal and in his coffee. Several of the cows in this herd which he milked had aborted in the summer and fall of 1950 and were known to have Bang's disease. An important point in the past history was that the patient had had rheumatic fever twice, once at the age of 13, and again at 20, while he was on active duty in the United States Navy, in 1944. He had been given a medical discharge from the Navy for rheumatic heart disease and was receiving partial disability benefits from the Veterans Administration.

Family history and functional inquiry were not contributory. Physical examination on admission revealed a well-developed well-nourished young white man lying quietly in bed, appearing acutely but not critically ill. His temperature was 101.8 F.; pulse rate, 80, and respiration rate, 22. There were three pinhead-sized petechiae on the buccal mucosa and one on the right elbow. Lymph nodes in the neck were slightly enlarged but not tender. There was marked desquamation of the toes and palms of the hands. The cardiovascular system findings were as follows: Pulse rate was 108; respiratory rate, 24; blood pressure, 110/56 to 0. The point of maximum impulse was felt 10 cm. from the midline in the fifth interspace. The second pulmonic sound was louder than the second aortic sound and was somewhat accentuated. There was a moderately loud, Grade 3, high-pitched blowing diastolic murmur, originating in the aortic area in the second right interspace along the right sternal border and transmitted down the left

sternal border. At the apex, with firm pressure on the bell of the stethoscope, a short early soft distant systolic murmur was heard. As pressure was released on the bell, a low-pitched rumbling presystolic murmur became audible. The rhythm was regular, with no sinus arrhythmia noted. The spleen was palpable 2 fingerbreadths below the left costal margin. The remainder of the physical findings were normal, except for evidence of dehydration.

Laboratory findings on admission were as follows: Urine, no abnormalities. Blood studies: hemoglobin, 14.2 gm. per 100 cc.; WBC, 5,400. Filamented polymorphonuclear neutrophiles, 75%; lymphocytes, 25%. Erythrocyte sedimentation rate, 32 mm. in an hour, corrected to 26 mm. in an hour; hematocrit level, 43%. The blood nonprotein nitrogen was 30 mg. per 100 cc. The highest white blood cell count recorded during the hospital stay was 10,700. Electrocardiograms were within normal limits. Full mouth x-rays revealed several carious teeth but no periapical infections. Sinus x-rays showed no abnormalities. Chest x-rays showed the cardiac silhouette to be somewhat aortic in configuration, with a suggestion of slight left auricular enlargement. A serologic test for syphilis was negative.

The clinical impression at this time was that the patient had rheumatic heart disease (probably inactive, but activity must be ruled out) with aortic insufficiency and mitral stenosis and insufficiency. Superimposed bacterial endocarditis seemed extremely likely. The possibility that the patient might have endocarditis due to one of the Brucella bacteria was considered. Brucellosis was also considered to be a strong possibility.



Agglutination titers of the patient's serum against Br. abortus and viridans Streptococcus.

During the first five days of hospitalization the patient had six severe bed-shaking chills, followed by high fever; on one occasion the temperature was as high as 106 F. During this period, six blood cultures were done. Each blood specimen was cultured in tryptose-phosphate broth and tryptose-phosphate-agar-yeast extract through which 10% CO_2 was bubbled. Five of these cultures were positive for Br. abortus, and two, taken on Jan. 16, 1951, were positive for viridans Streptococcus. This evidence indicated a bacteremia due to two different bacteria. A blood agglutination test performed the day after admission was positive in a dilution of 1:800 against Br. abortus but negative for typhoid, paratyphoid a and b, and tularemia. Throughout the patient's hospital stay the agglutination titers against Br. abortus remained consistently at 1:800. A skin test with brucellergin was strongly positive, and the skin lesion became necrotic. Intradermal tests with bacterial antigens (whole killed bacteria) made up of beta hemolytic streptococci, viridans Streptococcus, and nonhemolytic Streptococcus were performed three times and were always negative. Agglutination of the patient's serum against his own viridans Streptococcus was positive in a dilution of 1:4 initially, rose to 1:64, and fell to 1:4 again after recovery (Figure).

In view of the patient's condition, positive blood cultures for viridans Streptococcus, and an agglutination titer for Br. abortus of 1:800 (strong evidence in favor of brucellosis or endocarditis due to Br. abortus) it was planned to institute therapy with unusually large amounts of

antibiotics and chemotherapeutic agents. It was decided not to use penicillin because of the patient's recent bout with urticaria and exfoliative dermatitis following pencillin therapy. Results of sensitivity studies of the viridans Streptococcus cultured from the patient were as follows:

	Concentration Necessary to Inhibit Growth, γ/Ml.
Chlortetracycline	1.0
Chloramphenicol	5.0
Penicillin	0.3
Streptomycin	Resistant to 20

The treatment schedule outlined below was instituted on Jan. 19, 1951.

	Dose	Total Administered, Gm.	Duration of Administration, Days
Chlortetracycline	1 gm. q.4 h. for 11 days 1 gm. q.6 h. for 8 days 300 mg. q.2 h. for 5 days	116	24
Dihydrostreptomycin	0.5 gm. q.4 h.	36	10
Sulfadiazine	2 gm. q.4 h. with 1 gm. sodium bicarbonate q.4 h.	108	10

During the first week of hospitalization, parenteral fluids were administered daily in the form of 5% dextrose in isotonic sodium chloride solution. Ten days after treatment was begun painless gross hematuria occurred. Sulfadiazine and streptomycin were discontinued at this time, and the hematuria cleared.

Within two days after therapy was initiated the patient's temperature returned to normal, and it remained normal until Feb. 10, the 24th hospital day, when he began to complain of nausea and his temperature rose to a daily high of around 101.0 F. He vomited several times in the next few days, and on Feb. 11 the dose of chlortetracycline (Aureomycin) was reduced from 1 gm. every six hours to 300 mg. every two hours. The temperature remained elevated, but the nausea ceased. Chlortetracycline was discontinued on Feb. 16, and from that day on the patient's temperature remained normal and he improved steadily. Fourteen blood cultures taken after administration of antibiotic and chemotherapy were negative.

The patient was discharged well from the infirmary on Feb. 26, 40 days after admission. He graduated from the University and is employed as an auditor. He has continued to feel well and has regained strength. He notices no difference in exercise tolerance compared to that before his illness. He can play 9 holes of golf without noticing fatigue, but playing 18 holes leaves him tired. On July 21, 1951, a complete physical examination was performed. Cardiac findings suggested that slight enlargement had occurred since the patient had been discharged. The cardiac murmurs were the classic indications of aortic insufficiency and mitral stenosis and insufficiency. The agglutination titer for Br. abortus was 1:80; that with the patient's strain of viridans Streptococcus was 1:4, a year after recovery. In December, 1953, a card from the patient indicated that he was well.

COMMENT

On the basis of the patient's history, physical findings, clinical course, and bacteriologic and serologic findings (Figure) it would appear that the patient was actually infected by both viridans Streptococcus and Br. abortus and has recovered. Several possibilities exist in regard to the bacterial etiology of the endocarditis. First, he may have had endocarditis due to both organisms implanted on the endocardium. Second, he may have had brucellosis and endocarditis, with the endocarditis being due to viridans Streptococcus. Bacteremia in brucellosis without endocarditis is the rule. Neither the clinical nor the bacteriological findings furnish conclusive evidence as to which bacteria were implanted on the endocardium. Can help be obtained from the serological studies or skin tests? From the studies of Orgain and Poston [6] it would appear that in bacterial endocarditis opsonins, agglutinins, and bactericidins

are not present in significant titer in the patient's blood initially but that coincident with recovery they may reach a high titer and antibodies may develop coincident with disappearance of bacteremia.[17] The titer of antibodies usually diminishes after a few months. Kinsella [7] in 1917 reported that the serum of patients with subacute bacterial endocarditis contained agglutination antibodies against the homologous organism but not against heterologous strains recovered from patients with subacute bacterial endocarditis. Titers ranged between 1:10 to 1:40 and 1:10 to 1:140 in individual patients. Wright [8] observed that agglutination tests were positive in patients with subacute infective endocarditis in every case in dilutions of 1:10 to 1:320 but only with the homologous strain.

In brucellosis the agglutination titer is usually high during active infection and falls after recovery. In the reported cases of endocarditis due to Br. abortus the agglutination titers were universally high but response to the brucellergin skin test

Bacteriologic, Serologic, Skin Test, and Postmortem Findings in Nine Cases of Bacterial Endocarditis Due to Brucella

Reference	Blood Culture	Agglutination Titers	Skin Test	Postmortem Examination
9	Br. abortus	High	.....	"Brucella" observed in stained section of aortic valve
9	Br. abortus	High	.....	"Brucella" observed in stained section of mitral valve
10	Br. suis	1:640	Negative	Br. suis cultured from mitral valve
12	Br. melitensis	1:50-1:150	.....	Smear from mitral valve showed Gram-negative coccoid bacilli
14	Br. abortus	1:250	.....	Gram-negative bacilli from aortic valve; Br. abortus cultured from heart blood
15	Br. abortus	Br. abortus 1:9896 Br. melitensis 1:3766	.....	Bacteria showing Brucella morphology found in sections of mitral valve
1	Br. abortus	Br. abortus 1:1280 Br. melitensis 1:40 Br. suis 1:640	Brucellergin positive	Colonies of organisms in aortic valve vegetation
3	Negative	1:5120	.....	Br. melitensis cultured from aortic vegetation
4	Br. abortus	Br. melitensis Var. abortus 1:1280 Br. melitensis 1:500	Negative	Br. abortus cultured from fresh macerated valve vegetation

was inconsistent. The findings of the available blood cultures, agglutination titers, skin tests, and postmortem examination of nine cases of endocarditis reported to be caused by Brucella are shown in the Table.

Neither the agglutination titers nor the positive blood cultures are of much help in determining which bacteria were responsible for the endocarditis. Skin tests with streptococcal antigens were consistently negative, but the skin test with brucellergin was positive. The positive skin test with brucellergin in this case would probably mean the patient had active brucellosis, but this could hardly be used as evidence for or against endocarditis due to Br. abortus.

SUMMARY

A case has been presented of a patient with bacteremia due to viridans Streptococcus and Brucella abortus. It is probable that the patient had brucellosis with bacterial endocarditis due to viridans Streptococcus, although endocarditis due to both organisms cannot be ruled out. The patient recovered after therapy which included chlortetracycline (Aureomycin), streptomycin, and sulfadiazine and has remained well.

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News and Comment

ANNOUNCEMENTS

Convention of the Association of Military Surgeons.—The 61st Annual Convention of the Association of Military Surgeons of the United States will be held Nov. 29-30, Dec. 1, 1954, at the Hotel Statler, Washington, D. C. The program will cover a wide range of professional and scientific subjects which should be of interest to all those engaged in military medicine and the federal health services.

One of the outstanding features is the honors night dinner on Dec. 1, at which the Sir Henry Wellcome Medal and Prize, the Gorgas Medal, the Stitt Award, the McLester Award, the Louis Livingston Seaman Prize, and the Founder's Medal will be presented.

The granting of credit points for retirement to eligible reserve officers for attendance at meetings is expected to be announced soon by the department of defense.

Announcement of the Van Meter Prize Award.—The American Goiter Association again offers the Van Meter Prize Award of $300.00 and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The award will be made at the annual meeting of the association which will be held in Oklahoma City, April 28, 29, and 30, 1955, providing that essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed 3,000 words in length; must be presented in English, and a typewritten double space copy in duplicate sent to the Secretary, John C. McClintock, M.D., 149½ Washington Avenue, Albany, N. Y., not later than Jan. 15, 1955. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for the presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual proceedings of the association.

Hematology Fellowships.—The Division of Hematology of the Department of Medicine, University of Maryland School of Medicine, will have available as of July 1, 1955, two fellowships in hematology. These fellowships carry an annual stipend of $3,000.00 and provide training in clinical and research hematology. Inquiries should be addressed to Dr. Milton S. Sacks, University Hospital, Baltimore 1.

Medical Officer Examinations.—New examinations have been announced by the United States Civil Service Commission for filling Medical Officer positions in certain Federal agencies as follows: Positions of Medical Officer, paying $5,940 and $7,040 a year, and Medical Officer (Specialist), paying $8,360 to $10,800 a year, will be filled in various agencies in Washington, D. C., and vicinity, and in the United States Public Health Service and the Children's Bureau, located throughout the United States. Positions of Medical Officer, paying $7,425 to $10,450 a year, will be filled in the Panama Canal Company-Canal Zone Government Organization, in the Panama Canal Zone.

To qualify for these positions, applicants must be graduates of a medical school with the degree of doctor of medicine. For most of the Medical Officer positions at the entrance level, applicants must have completed a full internship. For Medical Officer (Specialist) positions, applicants must have completed appropriate study or residency training in the specialized field of medicine for which application is made. Professional medical experience is required for the higher-paying positions. No written test is required.

Full information regarding these positions is given in Announcement No. 415 for Medical Officer, Announcement No. 414(B) for Medical Officer in the Panama Canal Zone, and Examining Circular EC-27 for Medical Officer (Specialist). These notices may be consulted at most post offices, or copies may be secured direct from the United States Civil Service Commission,

Washington 25, D. C. Applications for all positions will be accepted until further notice. Applications for positions in agencies in the United States must be filed with the Commission's Washington Office. For positions in the Panama Canal Zone, send applications to the Board of United States Civil Service Examiners, Balboa Heights, Canal Zone.

Interpretation of Complex Arrhythmias.—A course in Interpretation of Complex Arrhythmias will be given at Michael Reese Hospital by Louis N. Katz, M.D.; Richard Langendorf, M.D., and Alfred Pick, M.D. This is an advanced course, intended only for experienced electrocardiographers. The class will meet daily from 9 a. m. to 5 p. m., Dec. 13-16.

Further information and a copy of the lecture schedule may be obtained from Secretary, Cardiovascular Department, Medical Research Institute, Michael Reese Hospital, Chicago 16.

Symposium on Chronic Pulmonary Disease.—A Symposium on Chronic Pulmonary Disease, planned especially for general medical practitioners, will take place Thursday, Nov. 11, 1954, in the building of the Medical Society of the County of Queens, 112-25 Queens Boulevard, Forest Hills, N. Y.

Sponsors are the Medical Society; the Queens County Chapter, Academy of General Practice, and the Queensboro Tuberculosis and Health Association.

The symposium will afford a comprehensive view of current concepts of the chronic pulmonary diseases, their diagnosis and treatment. It is approved by the American Academy of General Practice for five hours of formal credit.

The afternoon session begins at 2 p. m., and the evening session at 8 p. m.

For complete program information please call or write Miss Miriam Tauber, director, professional education, Queensboro Tuberculosis and Health Association, 159-29 90th Ave., Jamaica, New York, JAmaica 6-2557.

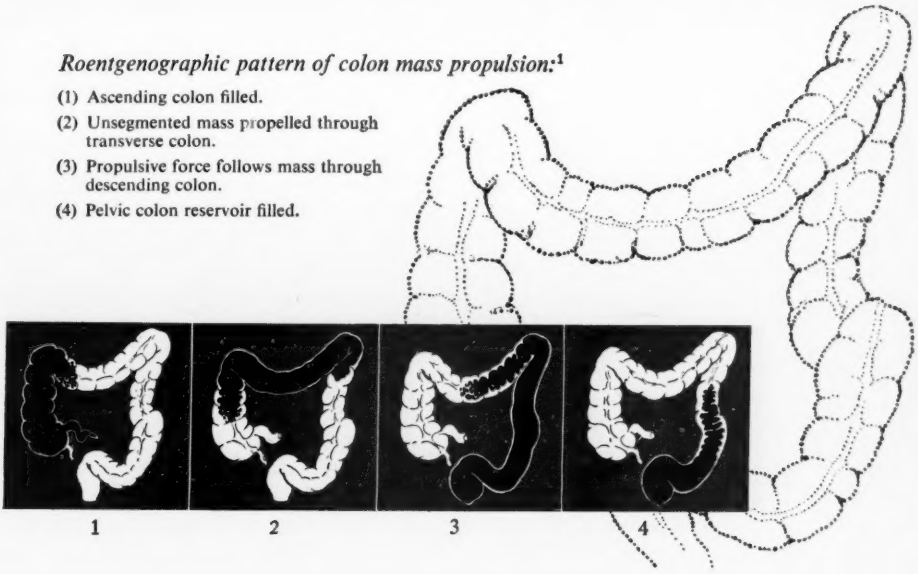
Immunization Information for International Travel.—A new revision of the booklet "Immunization Information for International Travel" has been released by the United States Public Health Service. Copies may be purchased from the Superintendent of Documents, Government Printing Office, Washington 25, D. C., at a cost of 20¢ per copy. There is a 25% discount allowed on orders of 100 or more delivered to the same address.

CORRECTION

Owing to a typographical error in the copy received for Table 2 of the article "Bishydroxycoumarin (Dicumerol) Therapy in Myocardial Infarction," by Drs. L. Feldman, G. L. Schaefer, S. E. Goldstein, and I. M. Forman, in the September issue (page 434), "anemia" was incorrectly substituted for "angina."

Roentgenographic pattern of colon mass propulsion:[1]

(1) Ascending colon filled.
(2) Unsegmented mass propelled through transverse colon.
(3) Propulsive force follows mass through descending colon.
(4) Pelvic colon reservoir filled.



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Lester M. Morrison, M.D., Los Angeles, in Rev. Gastroenterology, 20:744 (Oct.) 1953; abstract in J.A.M.A. 153:1580 (Dec. 26) 1953.

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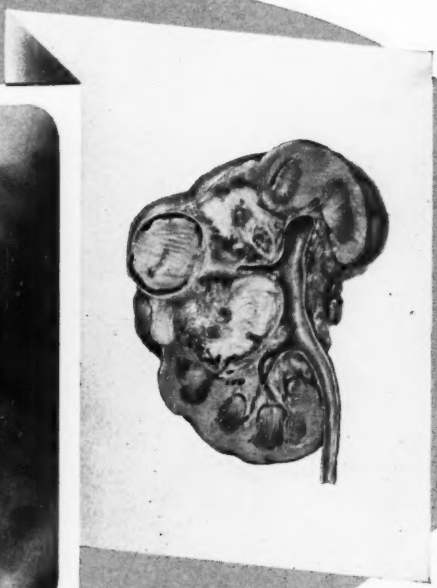

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C I B A

Summit, N. J.

2/2054 M